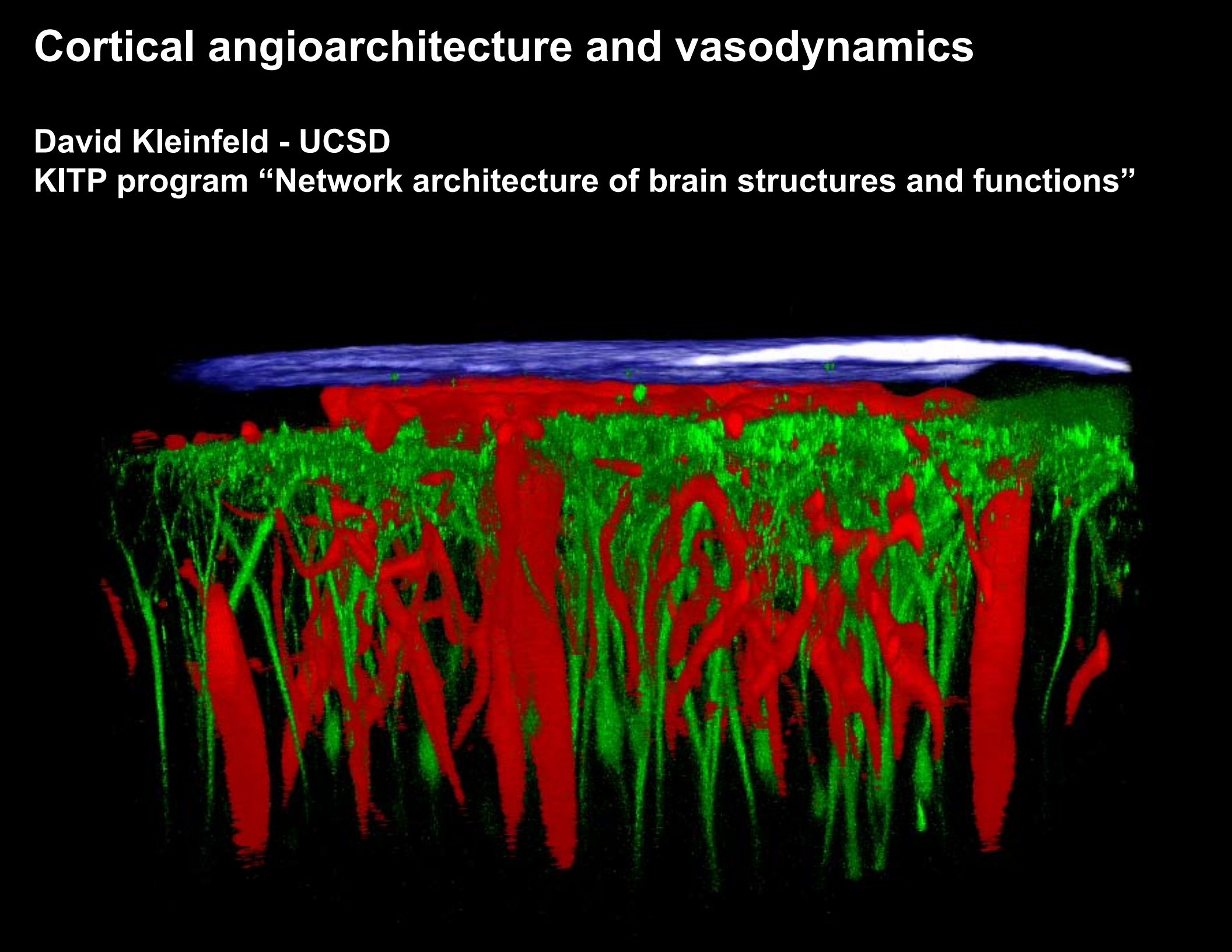
Cortical angioarchitecture and vasodynamics

David Kleinfeld - UCSD

KITP program "Network architecture of brain structures and functions"



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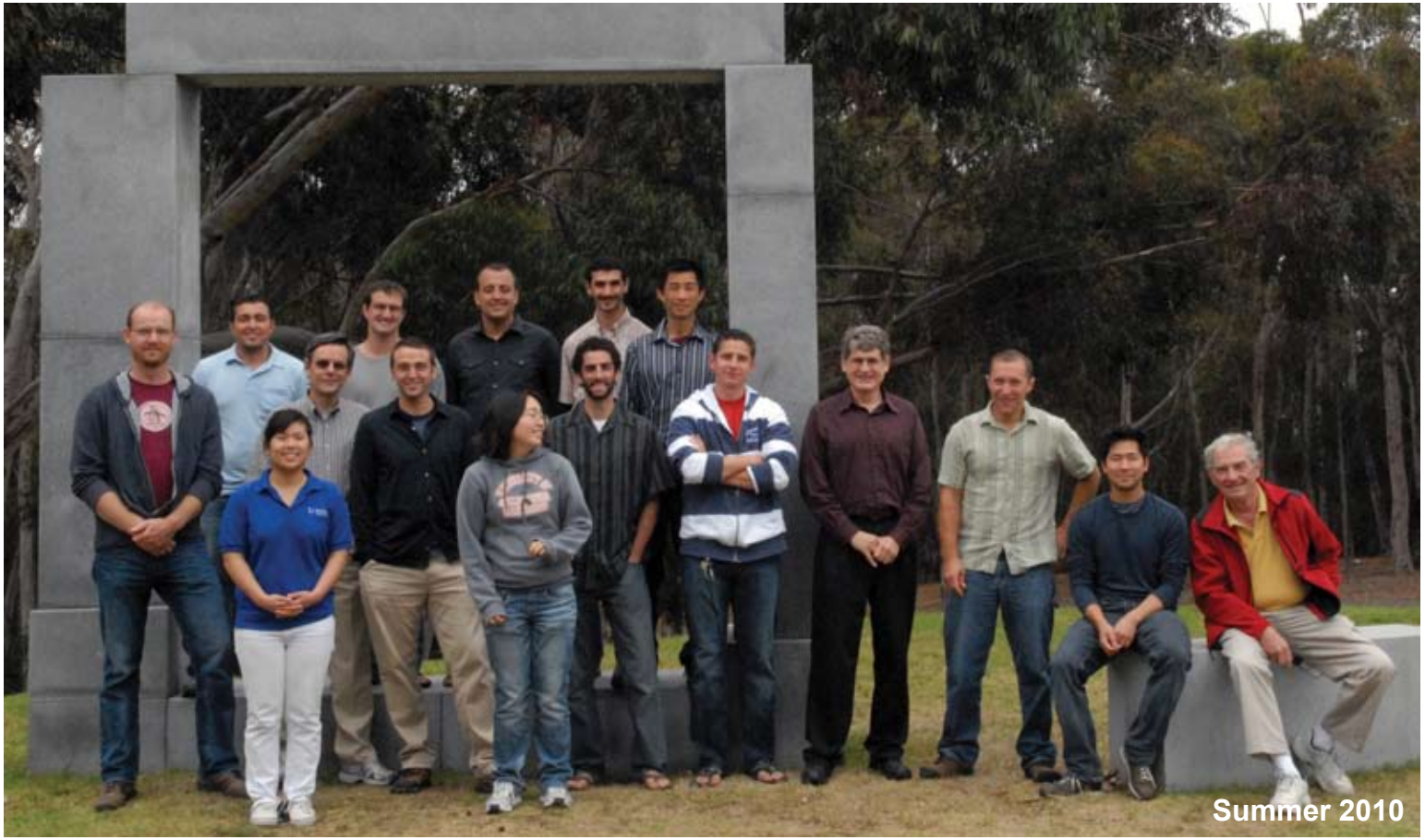

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Why should biologists be interested in cortical blood flow?

Dynamic resource allocation

Blood is a limited resource, yet neural function depends on adequate flow

Neuronal and vascular function are highly intertwined

Stroke and dysfunction of neurovascular control leads to neurological decline

Correspondence of blood flow and oxygenation to neural activity

Functional magnetic resonant imaging (fMRI) and intrinsic optical imaging (IOS) are powerful noninvasive tools to study brain function. Yet fMRI and IOS rely on poorly understood relationships between blood dynamics and neural activity.

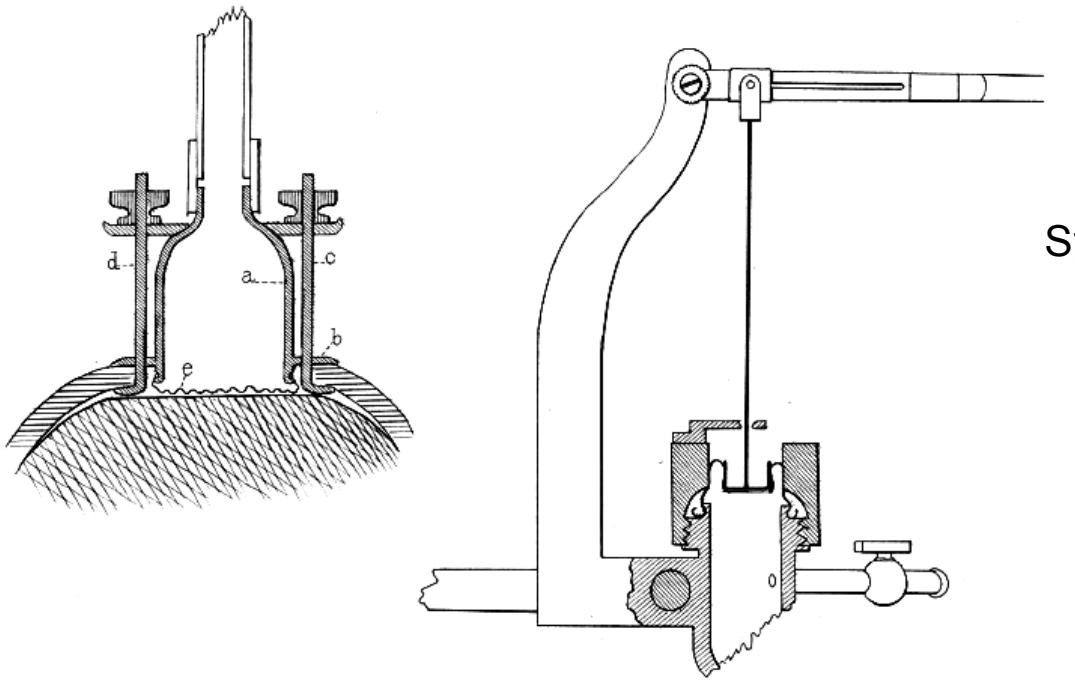
Our program

Connect network topology with the dynamics of flow

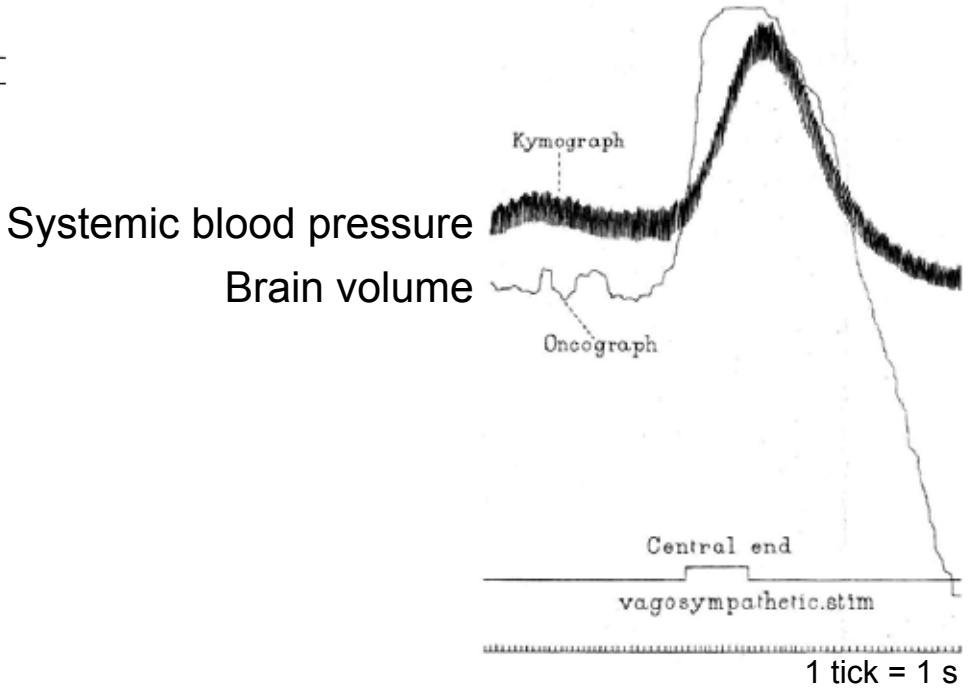
Understand the consequences of perturbation to flow

Understand the mechanism of neuronal control of flow

Earliest publication on vasodynamics in the mammalian brain (Roy & Sherrington, J Physiol 1890)

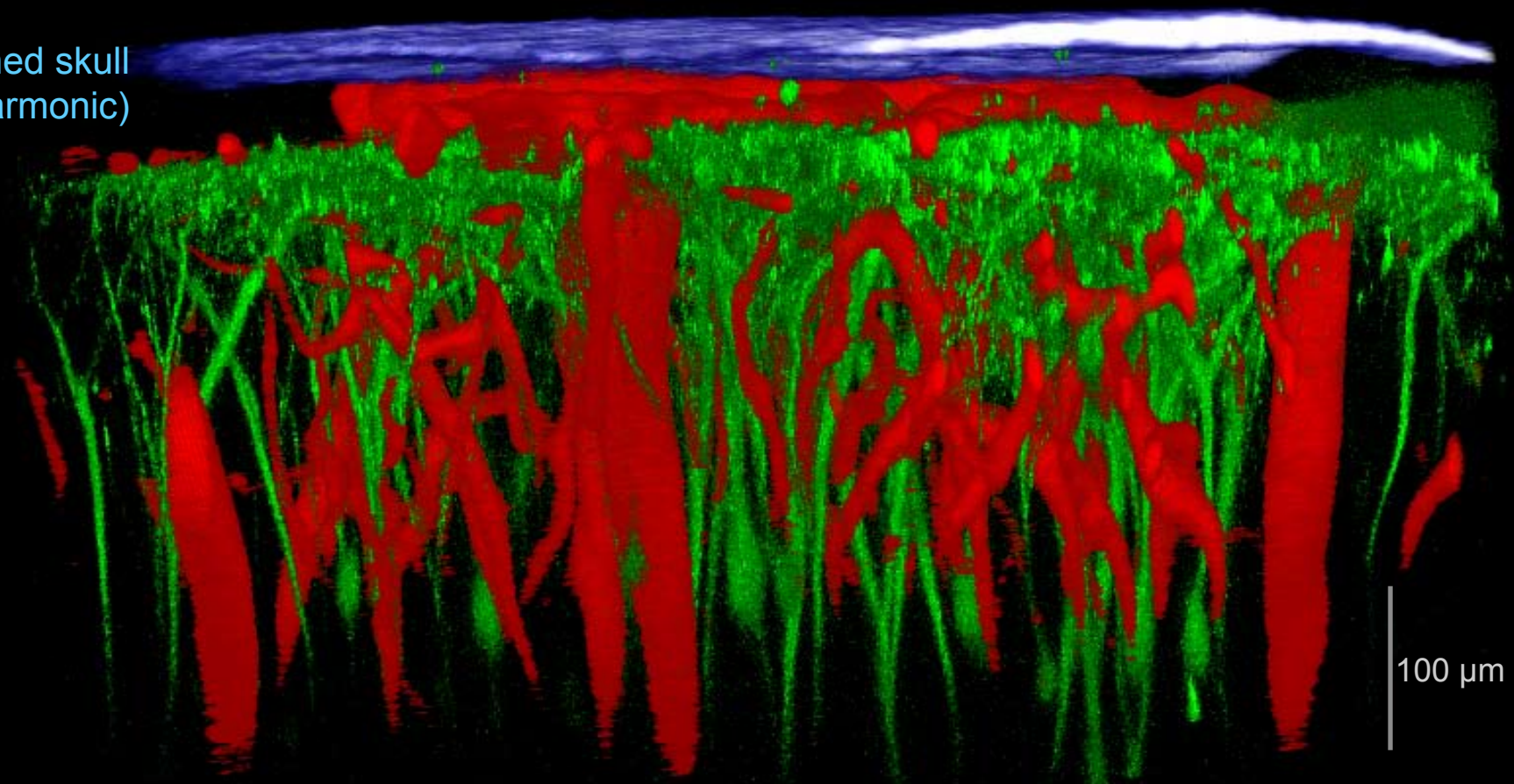


Sealed, constant pressure chamber
to record changes in brain volume



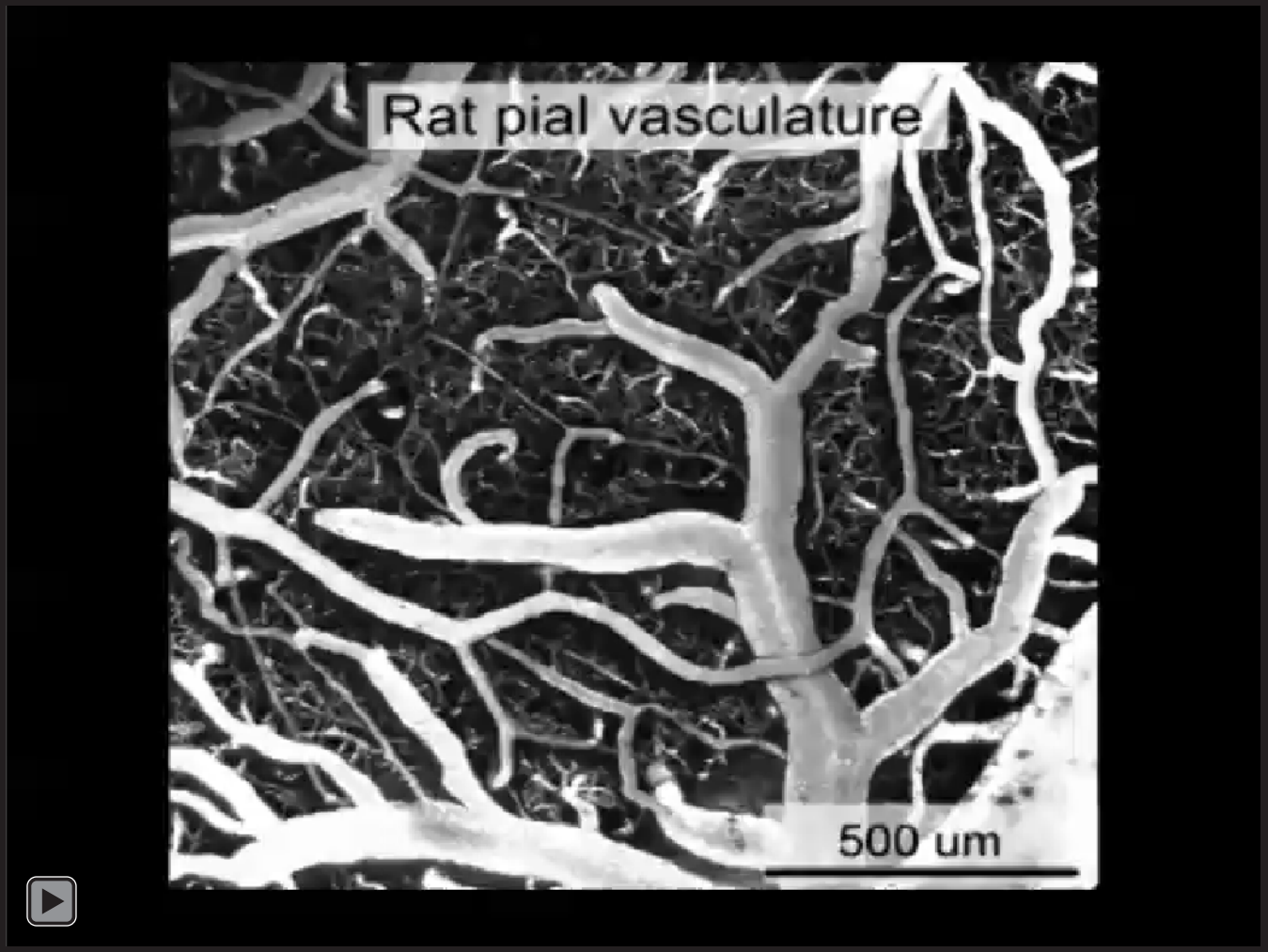
Changes in brain volume in response to
stimulation of the vagus (sympathetic nerve)

Polished and reinforced skull for chronic in vivo two-photon imaging



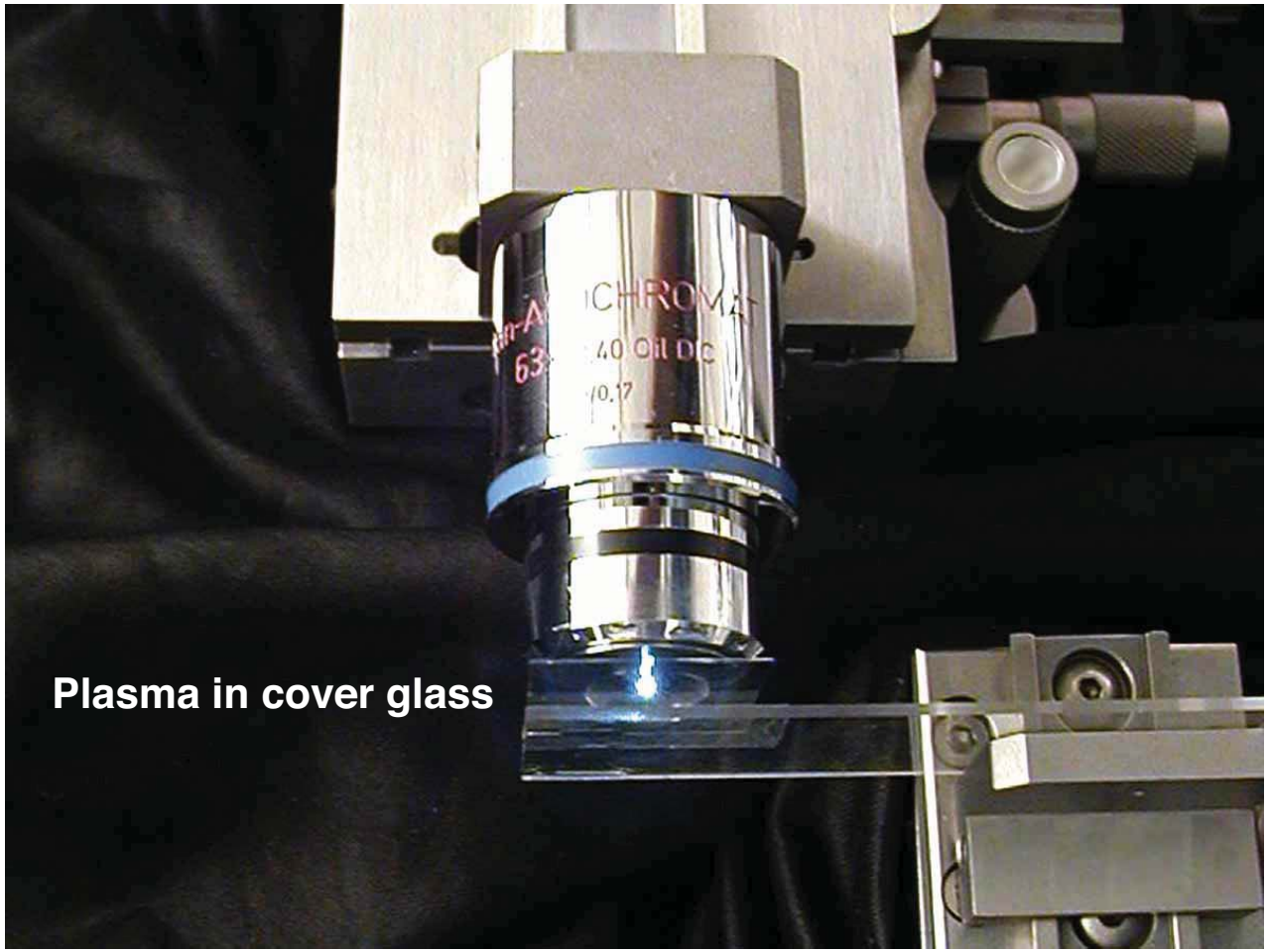
Drew, Shih, Driscoll, Knutsen, Blinder, Akassoglou, Davalos, Tsai & Kleinfeld (Nat Meth 2010)

Horizontal section of cortical vasculature via 2-photon microscopy



Movie by A. Y. Shih

Focused ultrashort laser pulses generate a plasma in materials

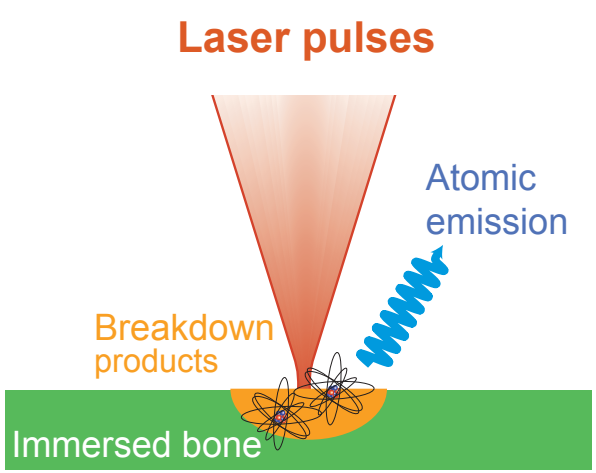


Schaffer, Brodeur, Garcia & Mazur (Optics Letters 2001)

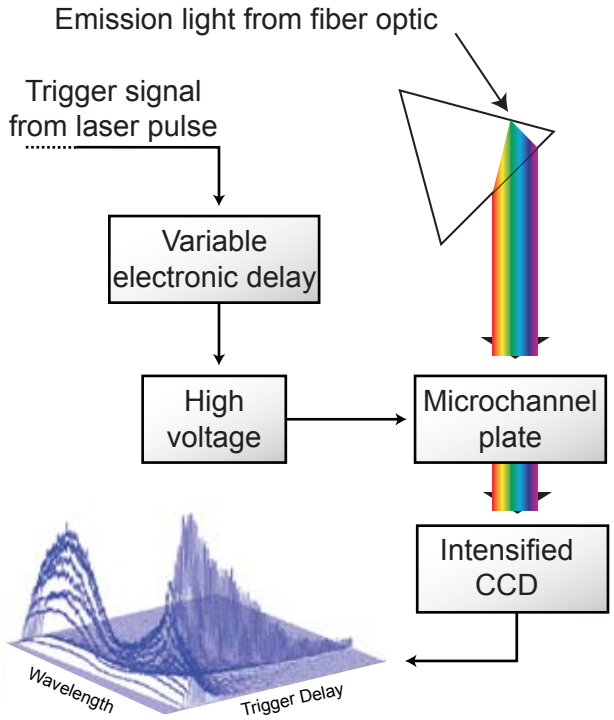
Loesel, Niemz, Nille & Juhasz (IEEE J Quantum Electronics 1996)

Stuart, Feit, Herman, Rubenchik, Shore & Perry (JOSA B 1996)

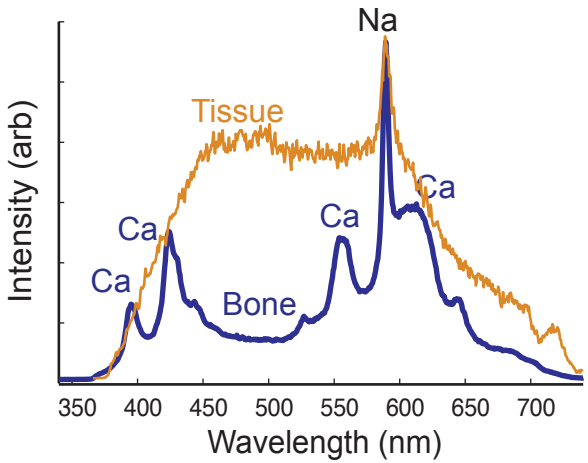
Laser induced plasma ablation and spectroscopy with amplified ultrafast pulses for automated surgery with feedback control



Time-resolved spectrum

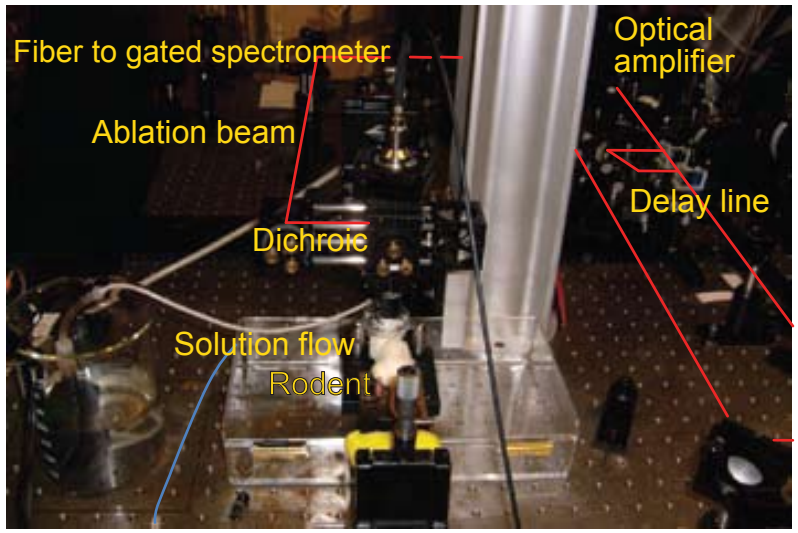


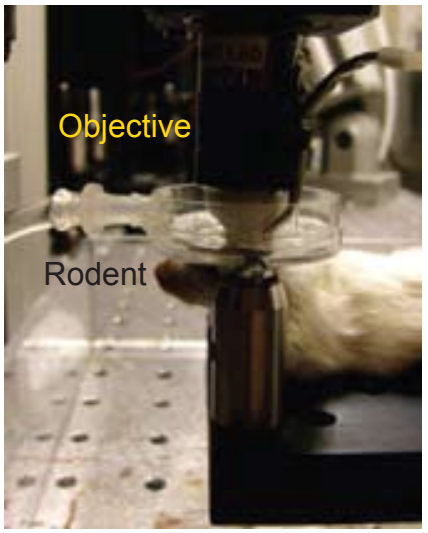
Delayed spectrum (20 ns)

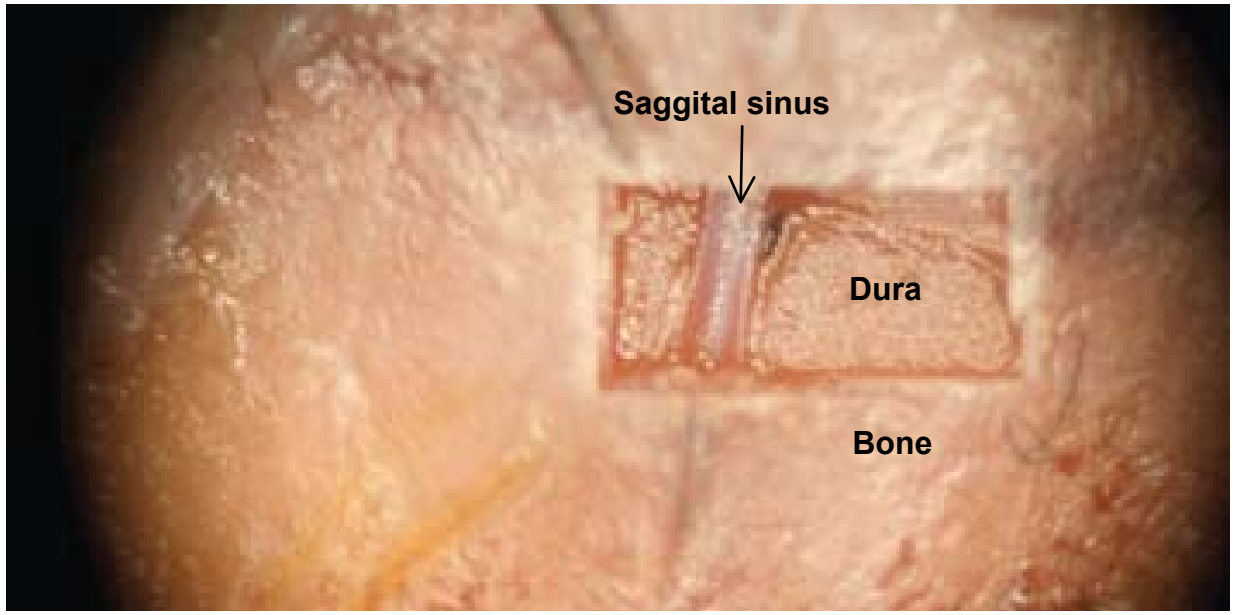


Jeong, Tsai, Shih, Schafer, Squier, Neev & Kleinfeld (SfN Abstract 2010)

All optical surgery with range finding and feedback (via LIPS and SHG)

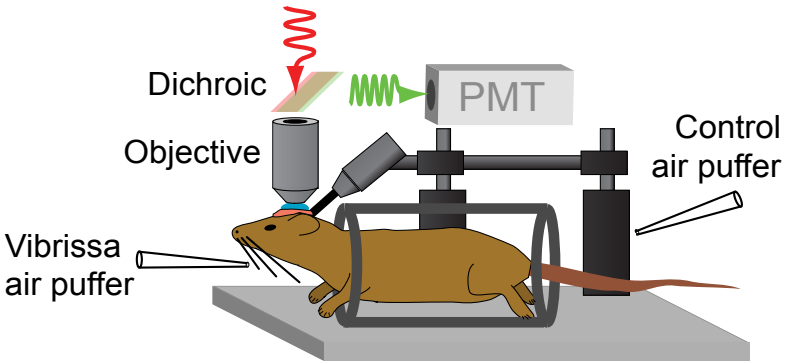


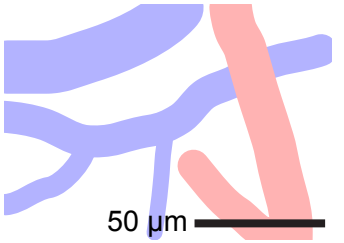


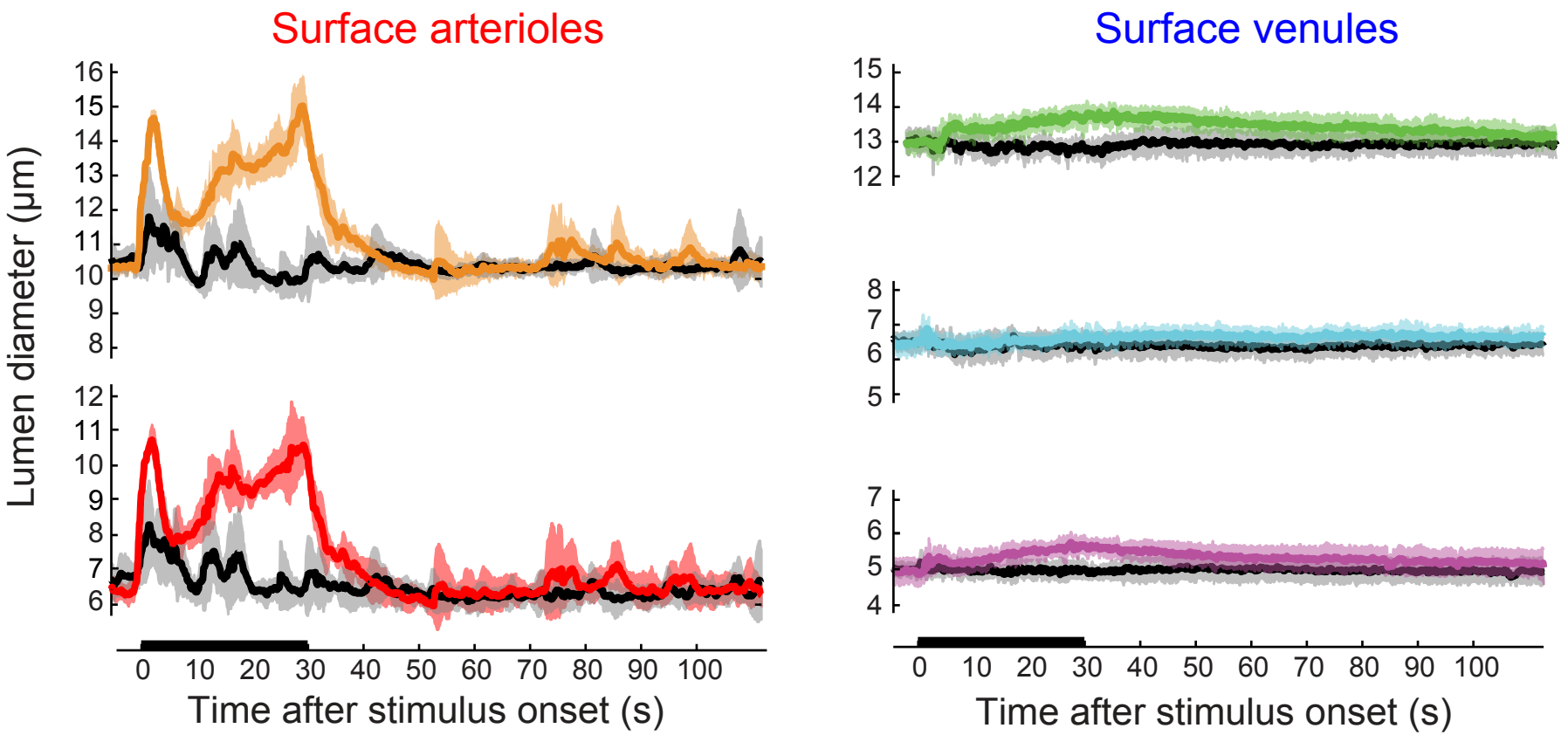


Jeong, Tsai, Shih, Schafer, Squier, Neev & Kleinfeld (SfN Abstract 2010)

Vascular changes in response to stimulation are predominantly arteriole

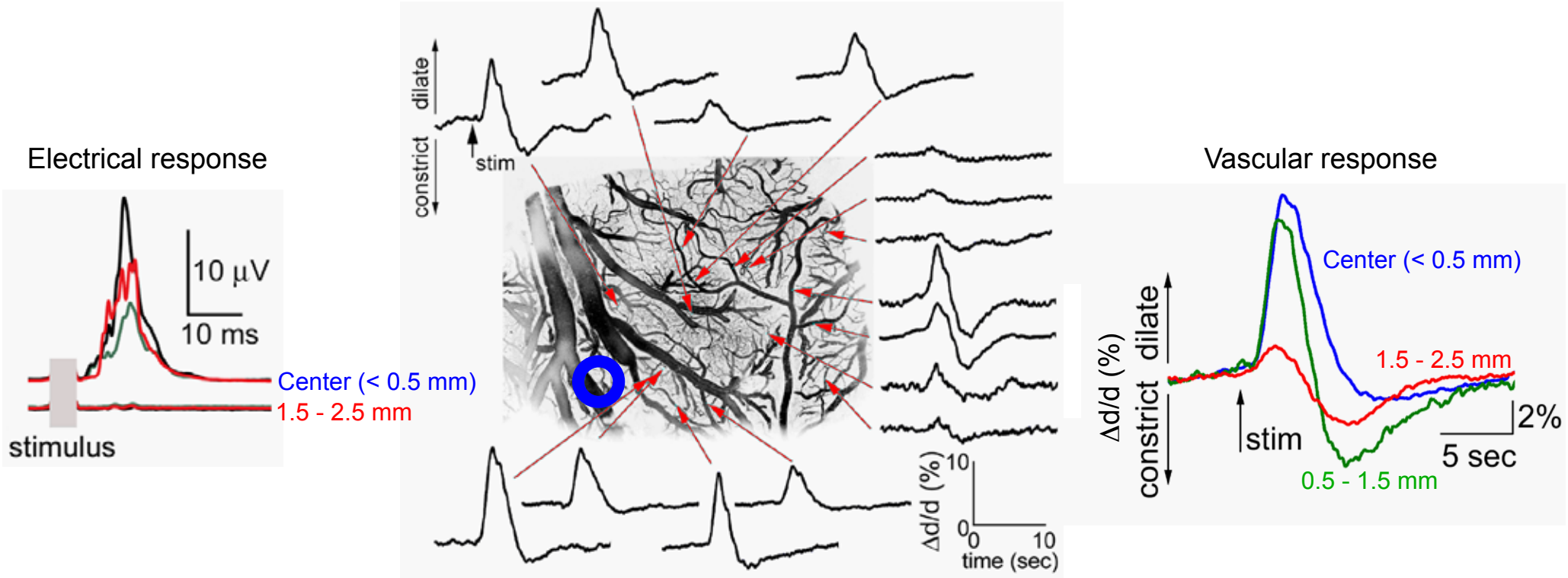






Drew, Shih & Kleinfeld (PNAS 2011)

Localized somatotopic stimulation leads to a center-surround pattern of vasodilation and vasoconstriction in contralateral surface arterioles

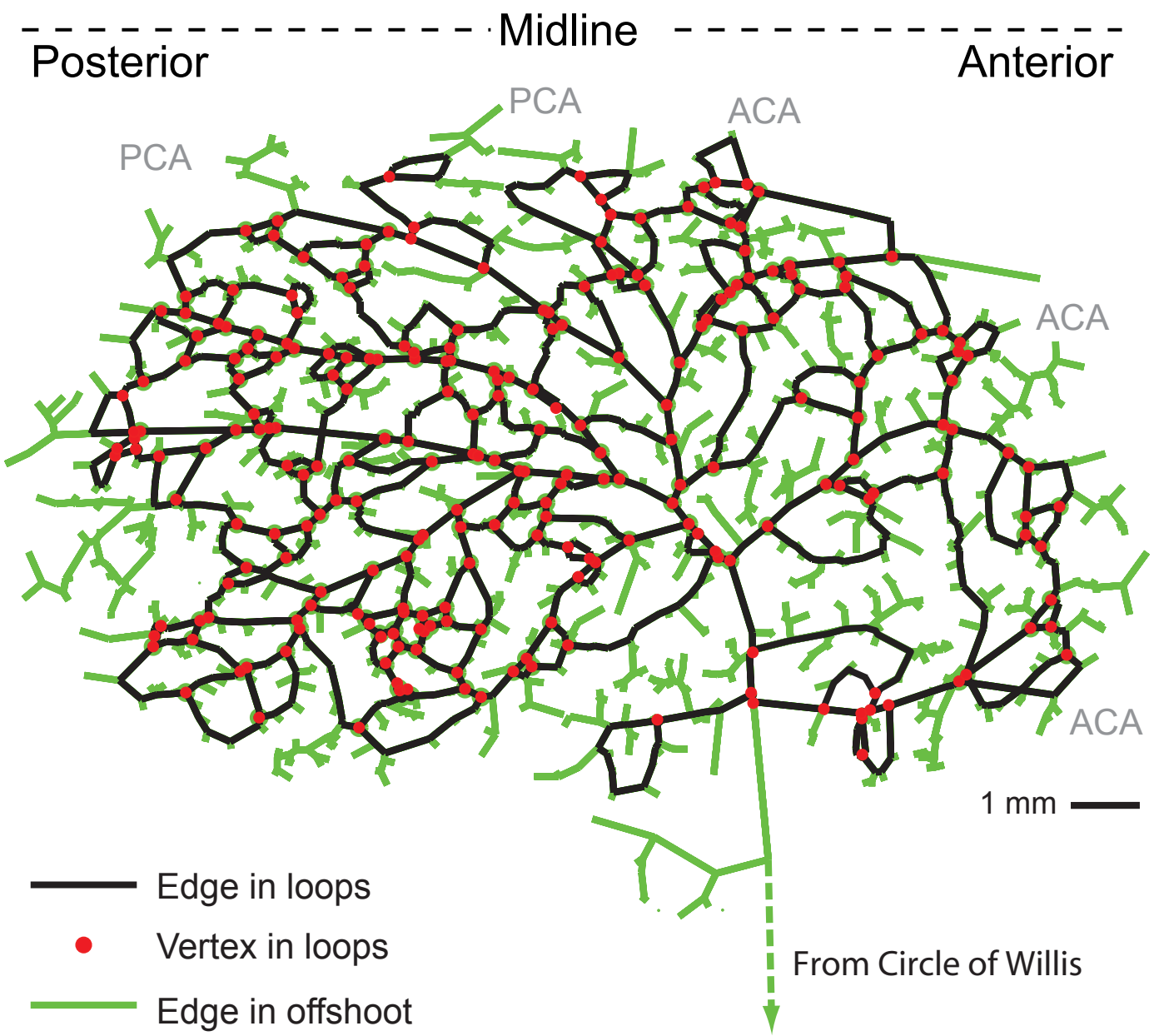


Devor, Tian, Nishimura, Teng, Hillman, Narayanan, Ulbert, Boas, Kleinfeld, Dale (J Neurosci 2007)

Lesson: Surface arterioles can redistribute blood to areas with heightened activity.

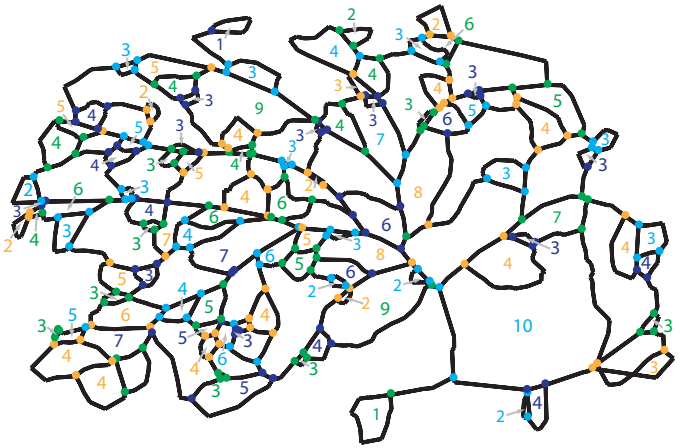
Hypothesis: Redistribution results from a highly interconnected network rather than a tree structure.

Surface arteriole network consists primarily of interconnected loops

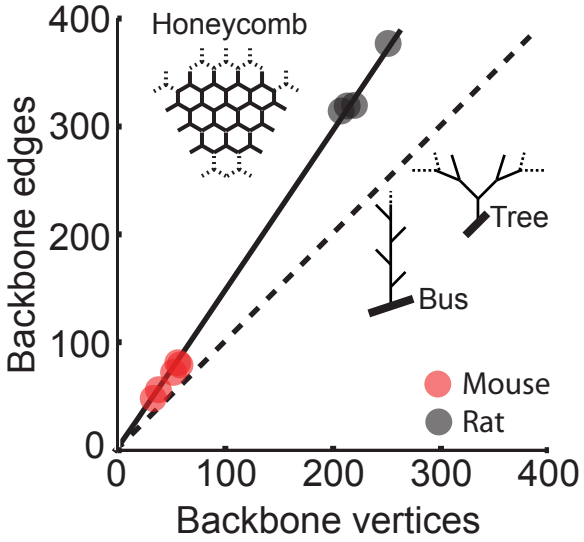


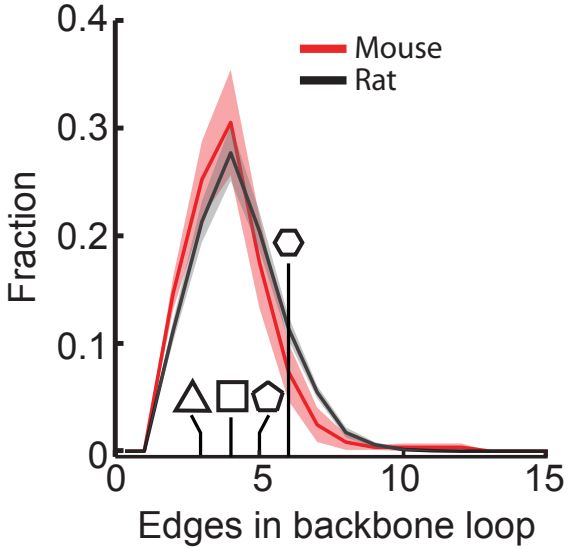
Blinder*, Shih*, Rafie & Kleinfeld (PNAS 2010)

Surface arteriole network consists primarily of interconnected loops



Kirchhoff-like backbone loops





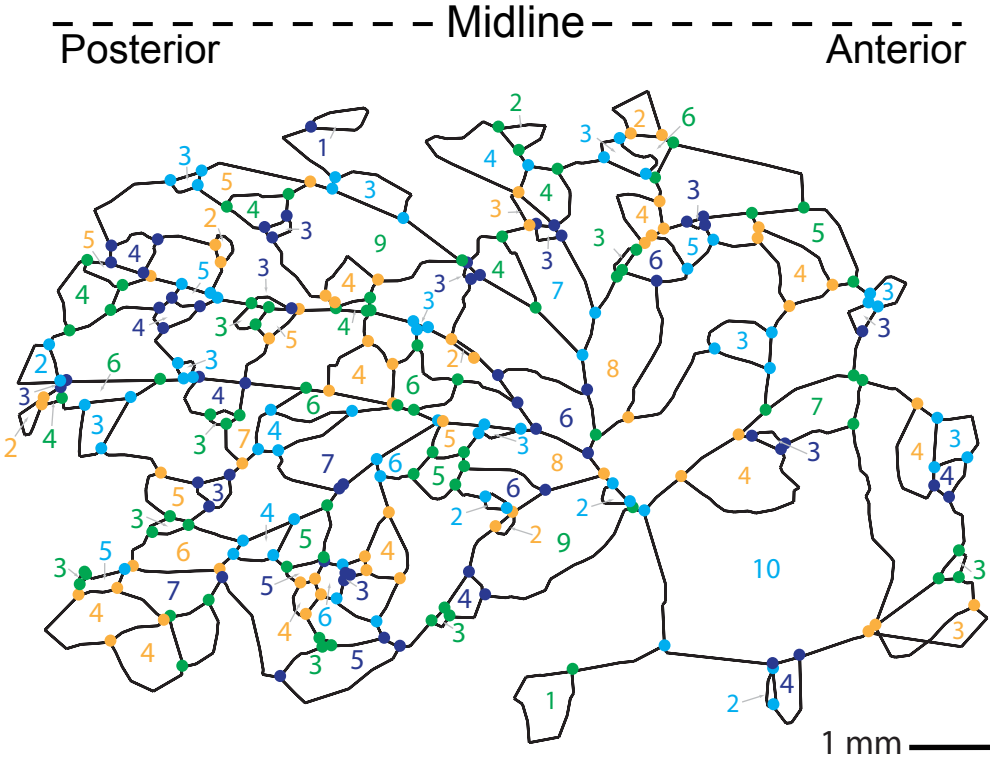
Coordination of 3 at *every* vertex - yet no simple lattice captures all features

Blinder*, Shih*, Rafie & Kleinfeld (PNAS 2010)

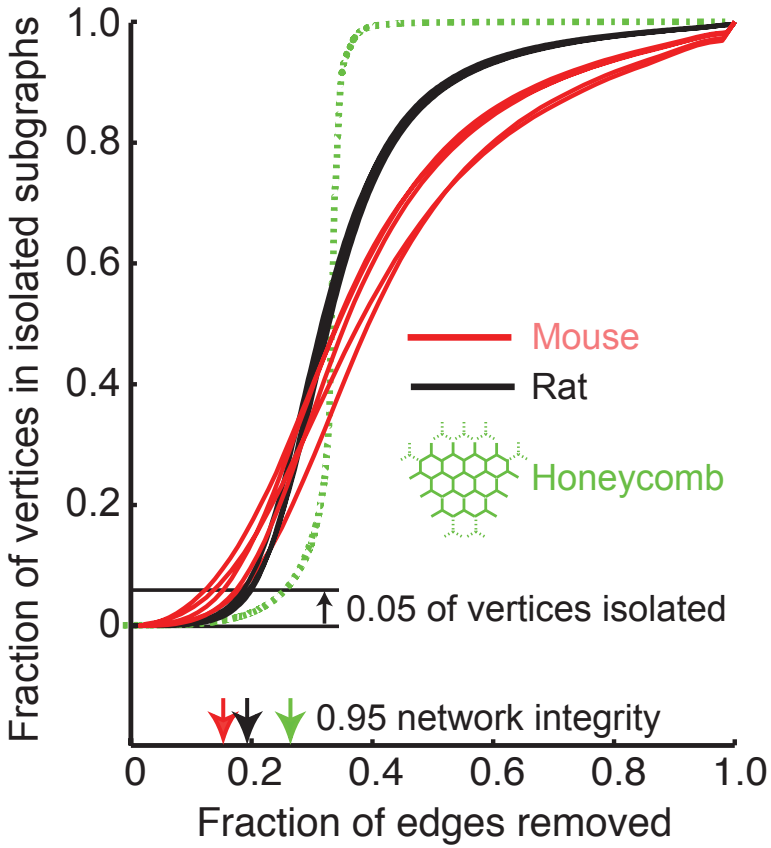
Lesson: Surface arterioles are arranged as a highly interconnected network.

Question: Does the surface angioarchitecture robustly circulate blood?

Calculations support the robust nature of the surface network



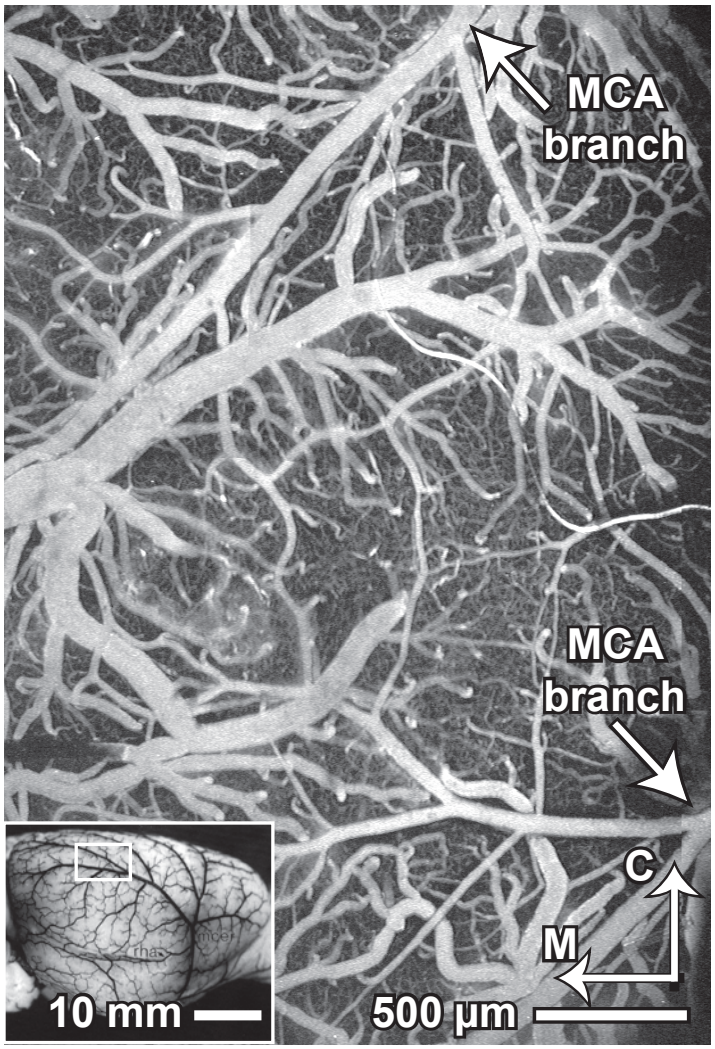
Kirchhoff-like backbone loops

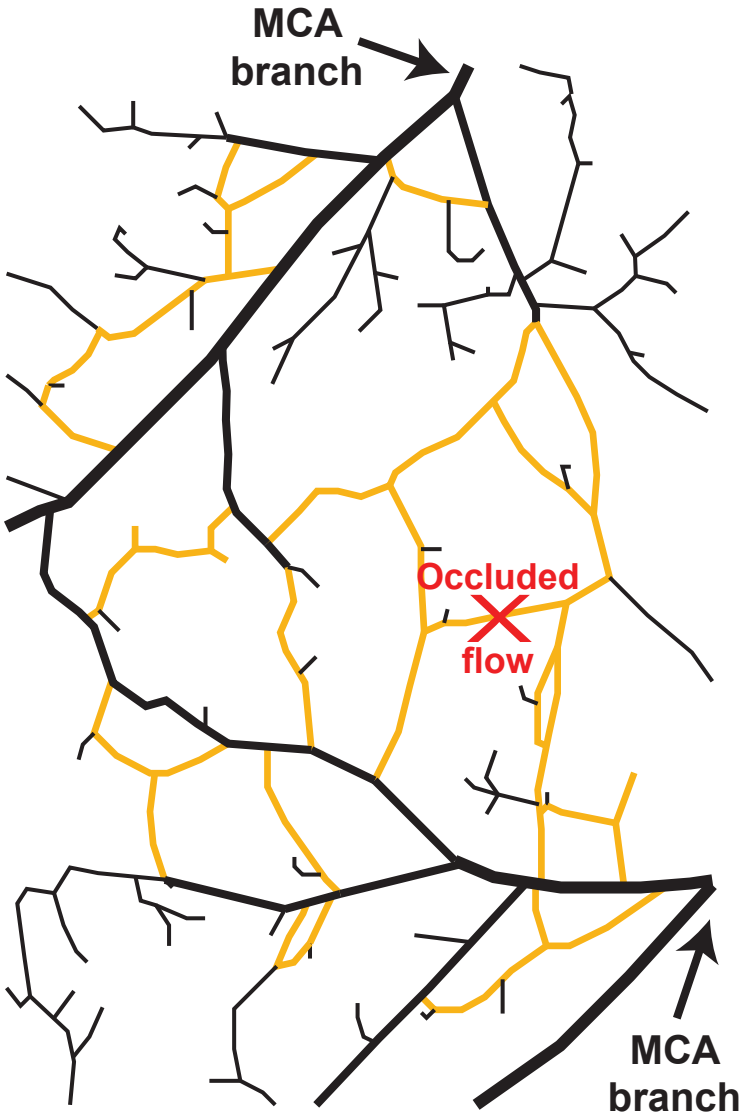


Prediction: Edges may be removed with minimal consequence to overall flow

Blinder*, Shih*, Rafie & Kleinfeld (PNAS 2010)

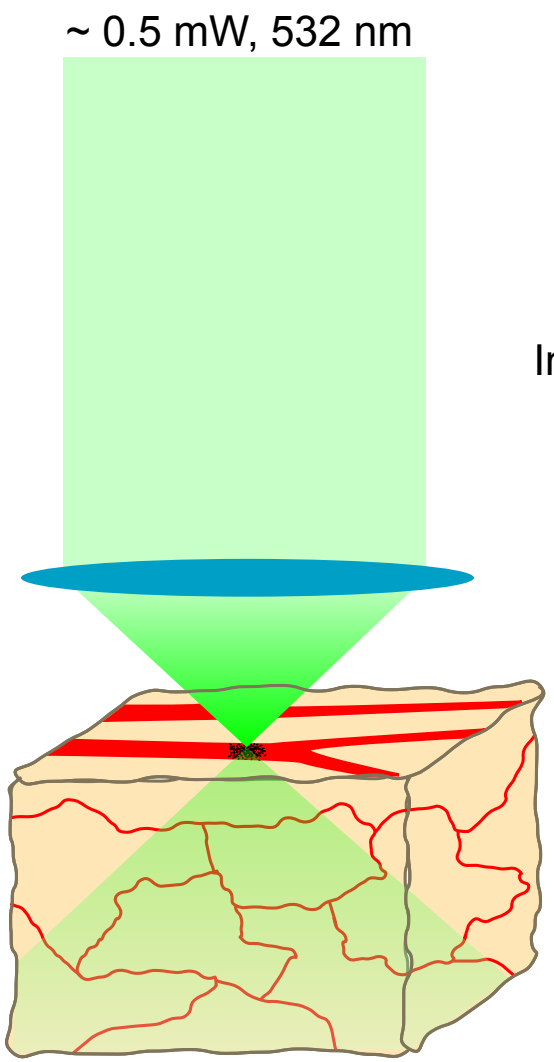
Two-photon microscopy to image vasculature and target an occlusion





Schaffer, Friedman, Nishimura, Schroeder, Tsai, Ebner, Lyden & Kleinfeld (PLoS Biology 2006)

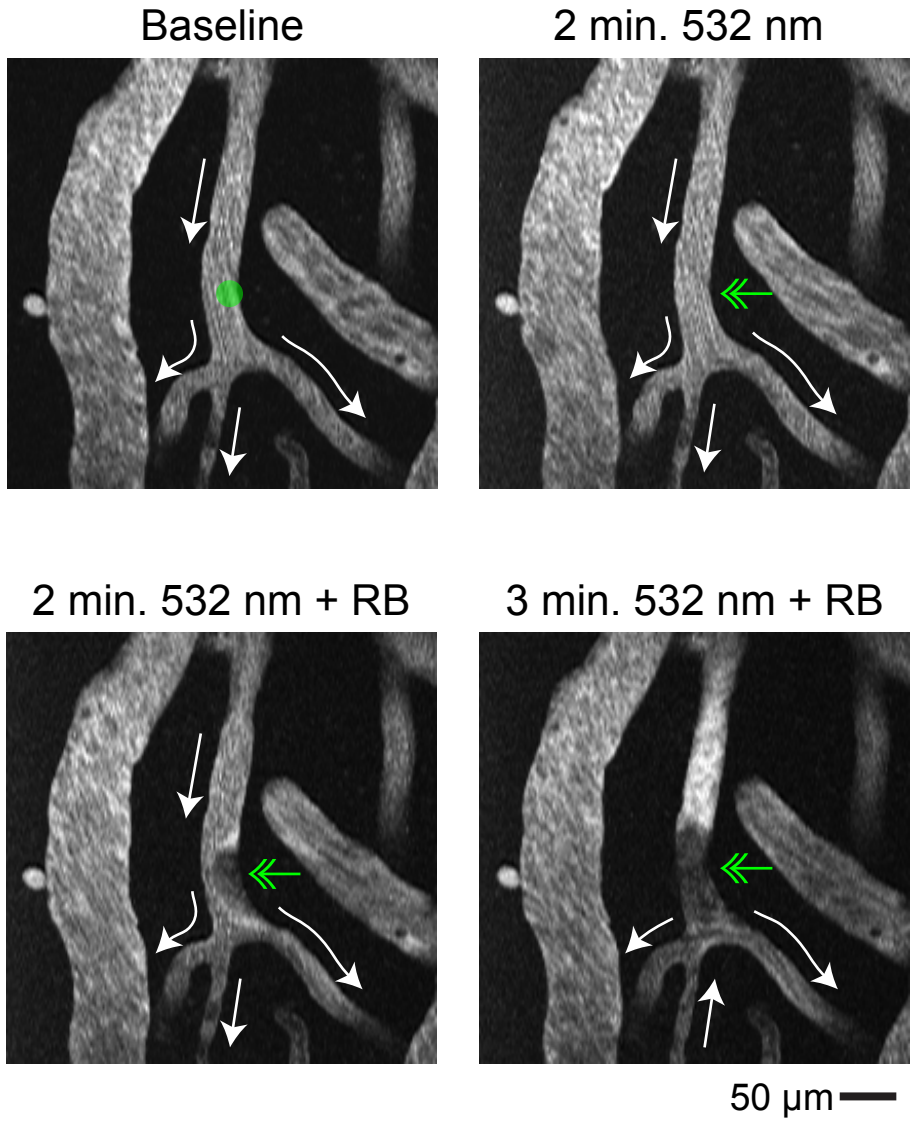
Targeted photothrombotic occlusion to a selected surface vessel



Target vessel of interest

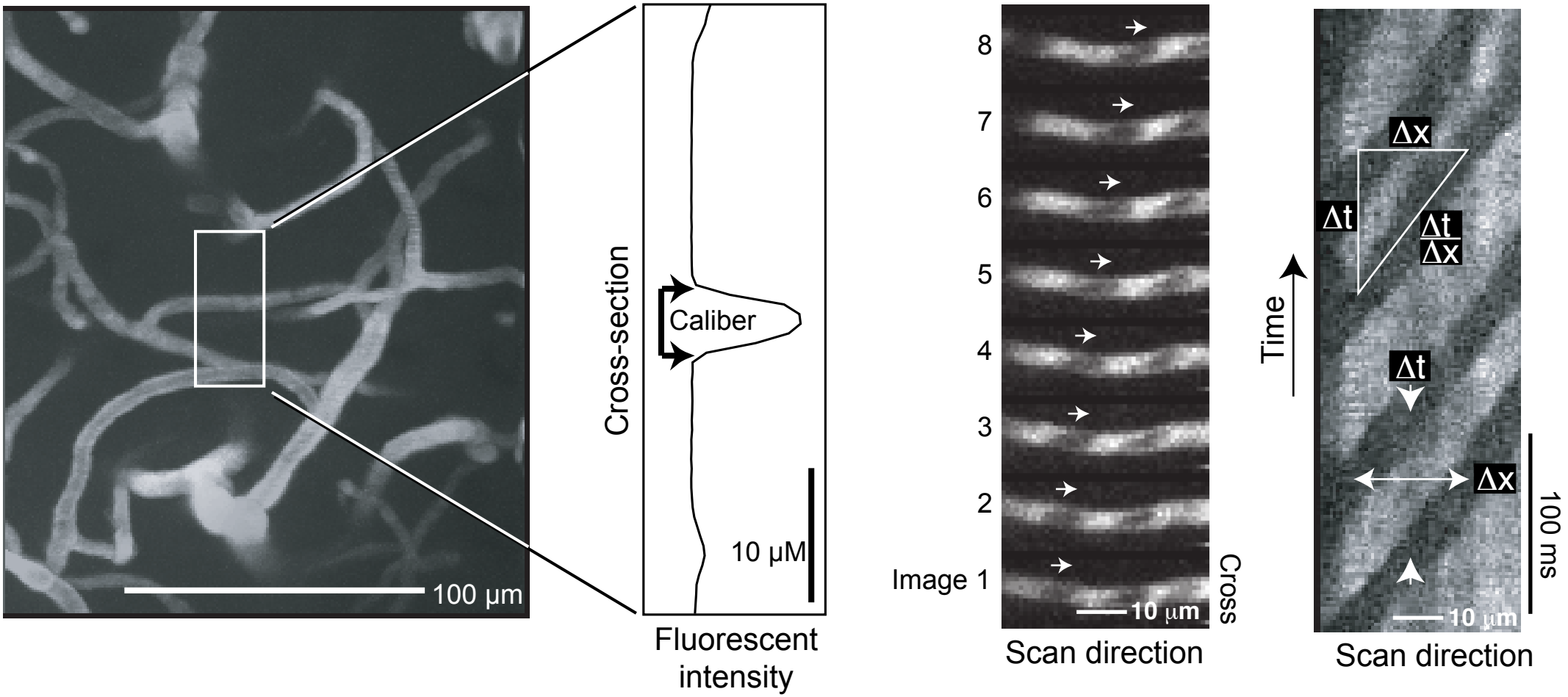
Inject Rose Bengal (~5 min half life)

Clot targeted surface vessel



Schaffer, Friedman, Nishimura, Schroeder, Tsai, Ebner, Lyden & Kleinfeld (PLoS Biology 2006)

In vivo two-photon microscopy for red blood cell tracking

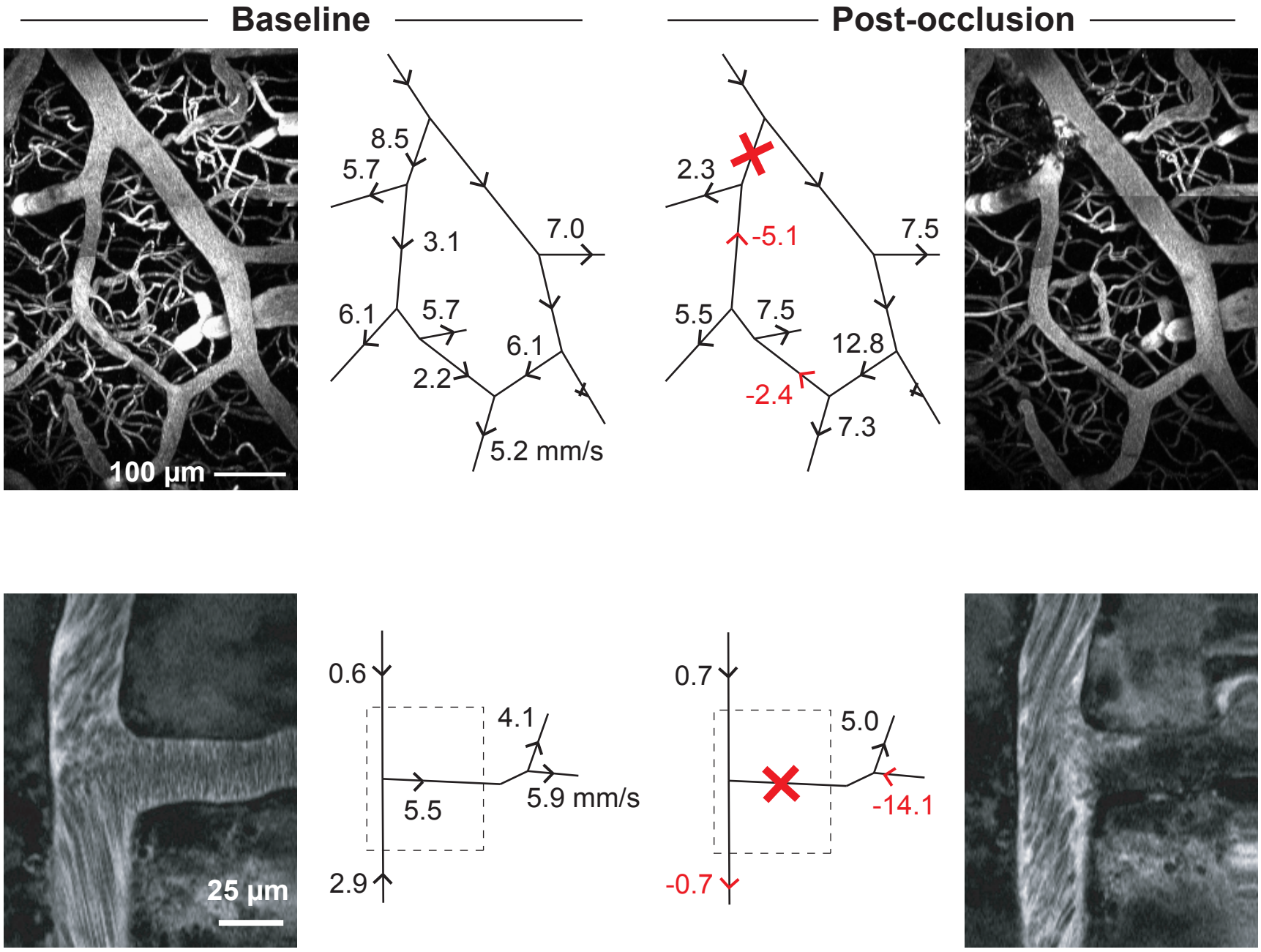


Automatic calculation of speed $v = (\Delta t/\Delta x)^{-1}$

Kleinfeld, Helmchen, Mitra & Denk (1998)

Drew, Blinder, Cauwenberghs, Shih & Kleinfeld (J Comp Neurosci 2010)

Reversal of RBC flow in downstream arterioles after targeted occlusion

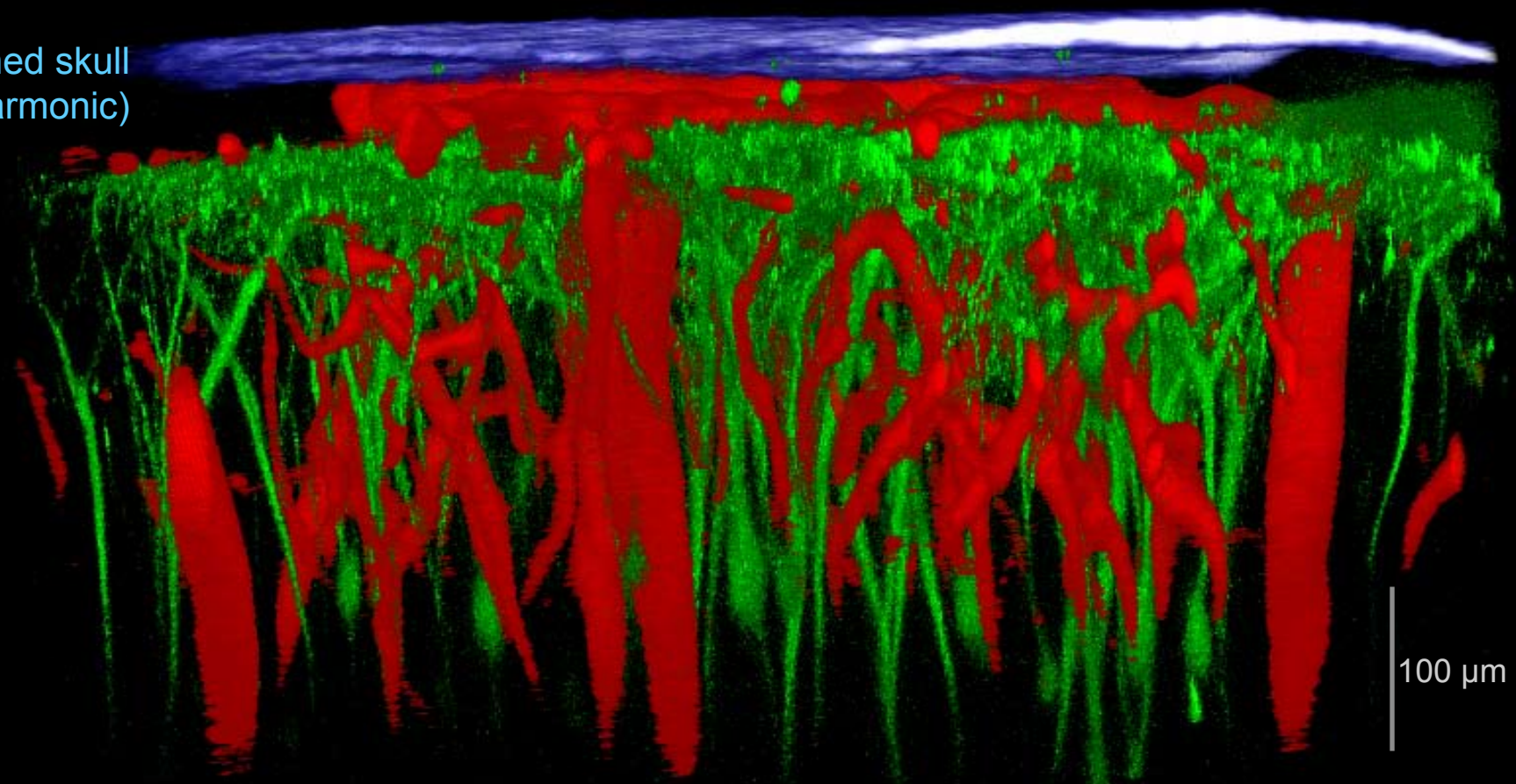


Schaffer, Friedman, Nishimura, Schroeder, Tsai, Ebner, Lyden, Kleinfeld (PLoS Biology 2006)

Lesson: Blood flow in the surface arteriole network is robust.

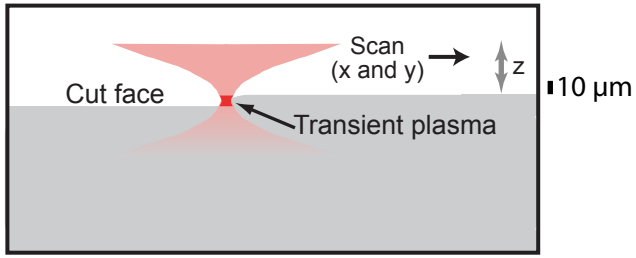
We turn our attention to the routing of blood from the surface network to the subsurface microvasculature.

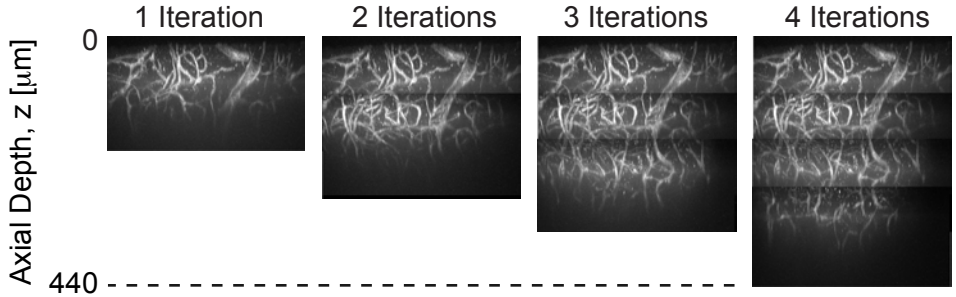
Polished and reinforced skull for chronic in vivo two-photon imaging



Drew, Shih, Driscoll, Knutsen, Blinder, Akassoglou, Davalos, Tsai & Kleinfeld (Nat Meth 2010)

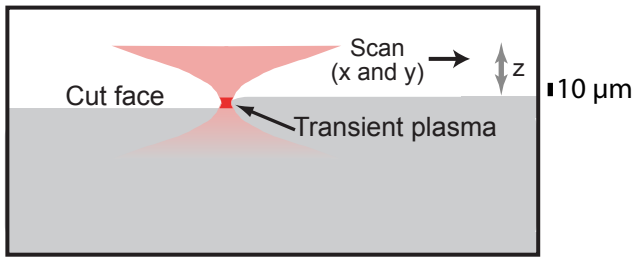
All optical histology and automated tracing for vectorized anatomy

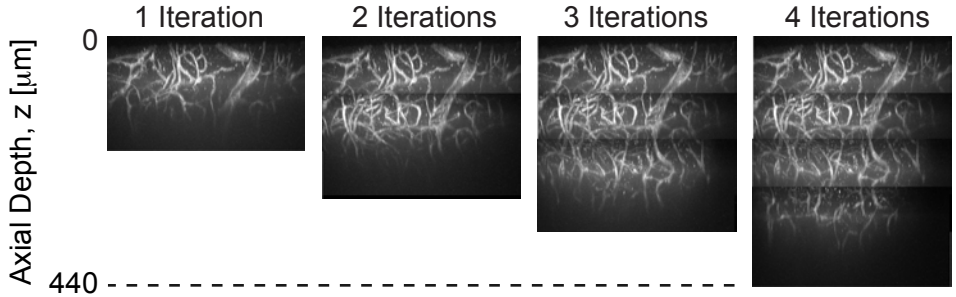




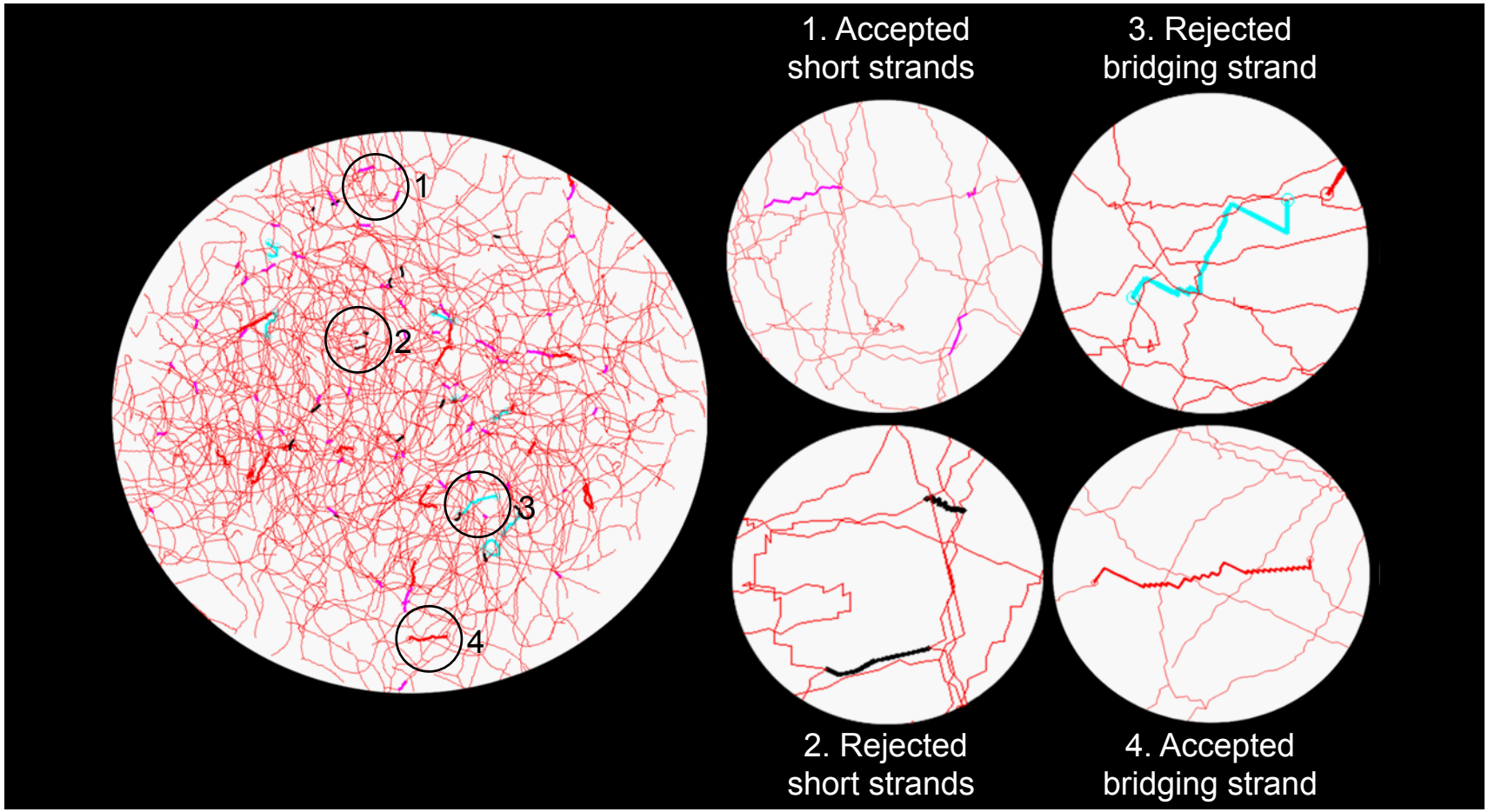
Tsai, Friedman, Ifarraguerri, Thompson, Lev-Ram, Schaffer, Xiong, Tsien, Squier and Kleinfeld (Neuron 2003)

All optical histology and automated tracing for vectorized anatomy



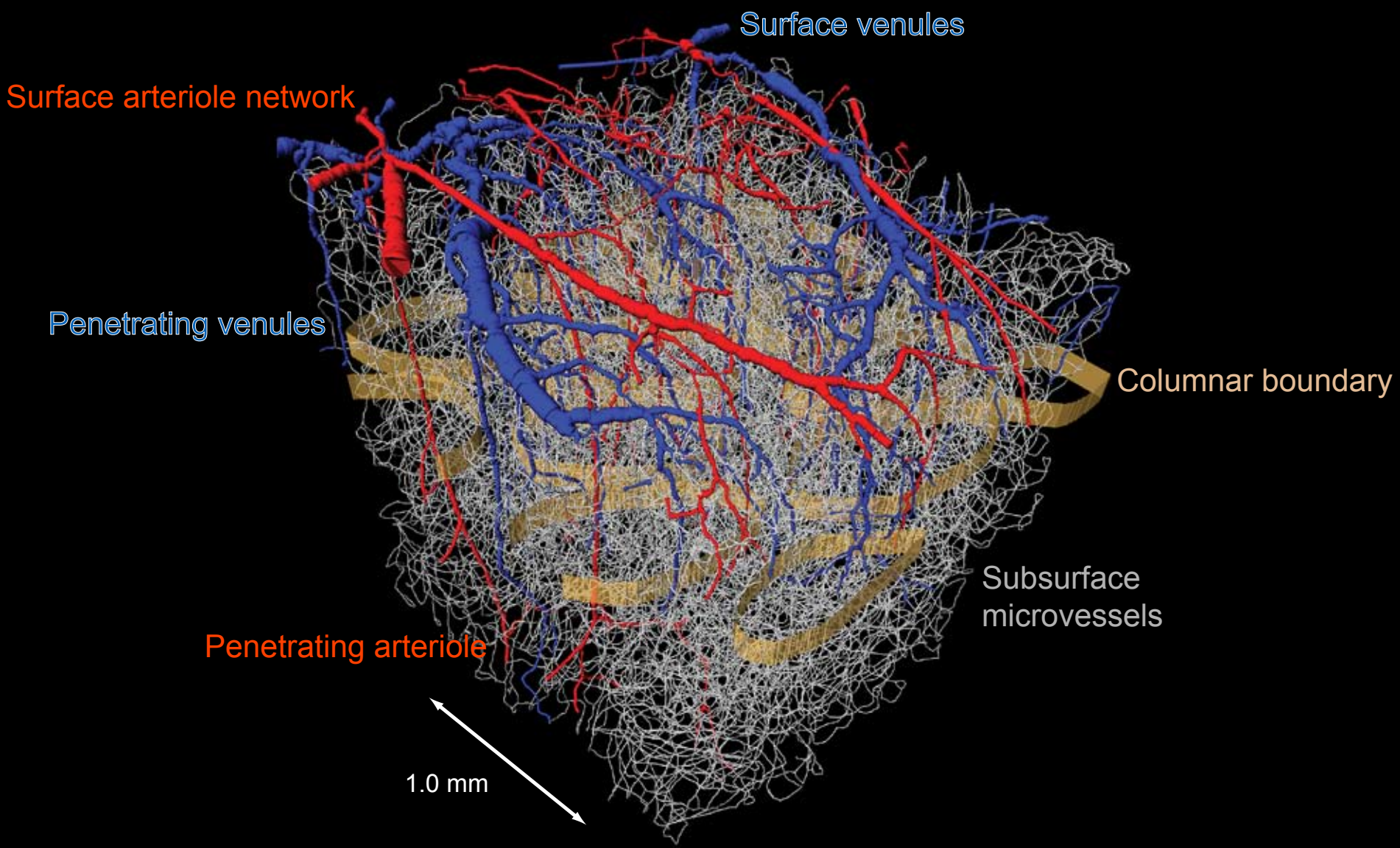


Tsai, Friedman, Ifarraguerri, Thompson, Lev-Ram, Schaffer, Xiong, Tsien, Squier and Kleinfeld (Neuron 2003)



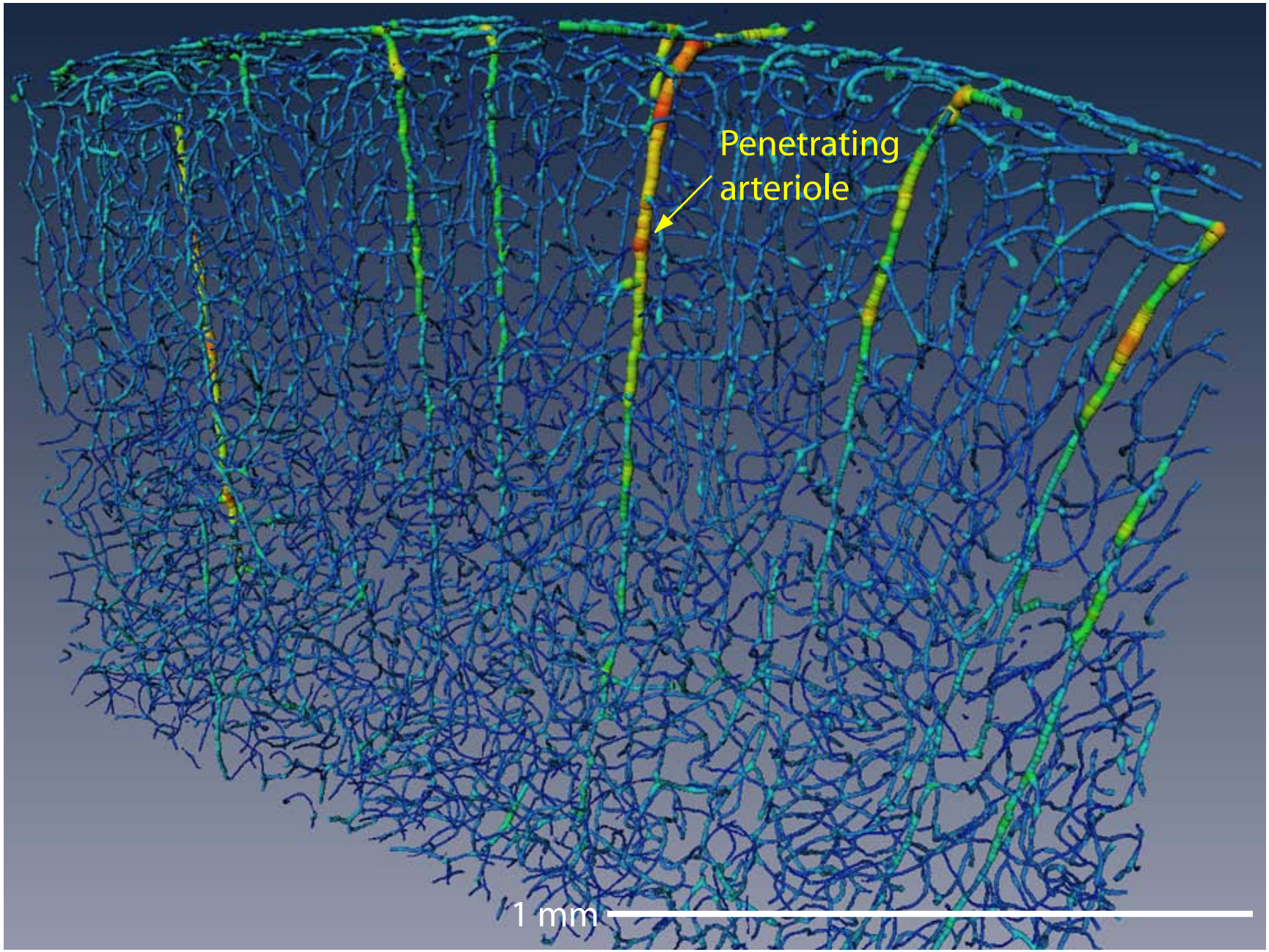
Kaufhold, Tsai, Blinder & Kleinfeld (MIAAB/MICCAI 2008; full paper submitted)

Features of cortical vasculature from a 2 mm^3 angiotome



Blinder*, Tsai*, Kaufhold & Kleinfeld (SfN abstract 2010)

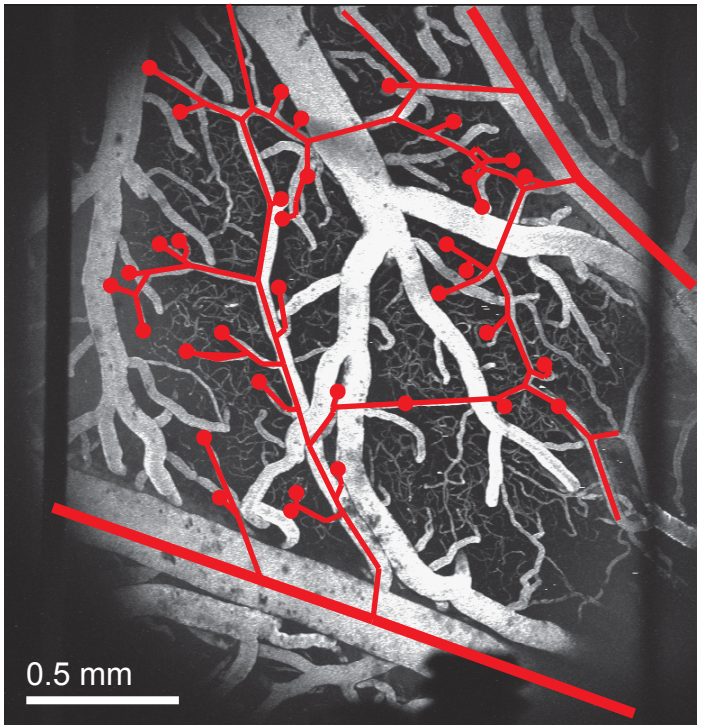
Penetrating arterioles deliver blood to subsurface microvessels

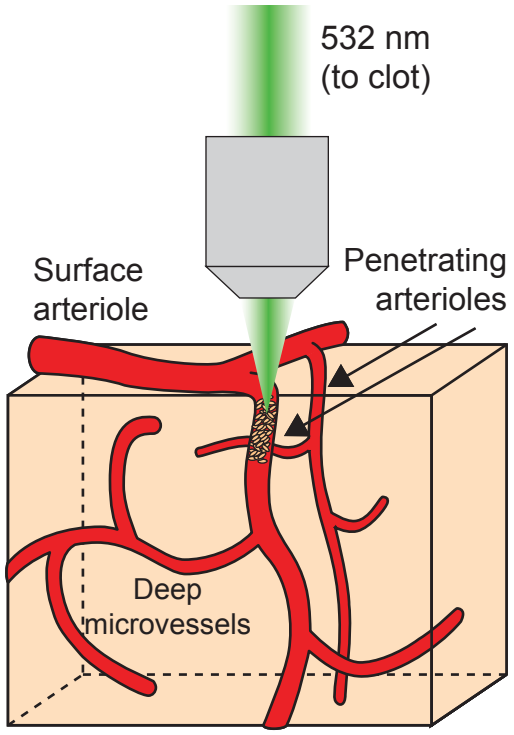


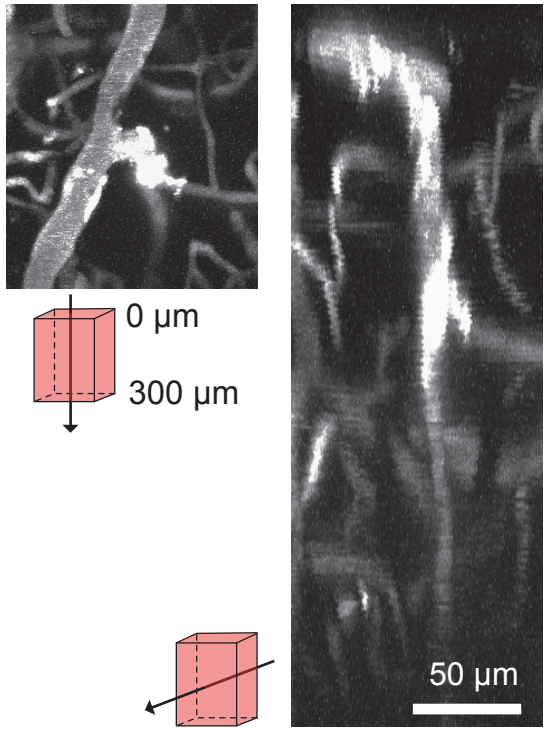
Blinder*, Tsai*, Kaufhold & Kleinfeld (SfN abstract 2010)

Question: Is there collateral flow between neighboring penetrating arterioles?

TPLSM to target photothrombotic occlusion to a penetrating arteriole



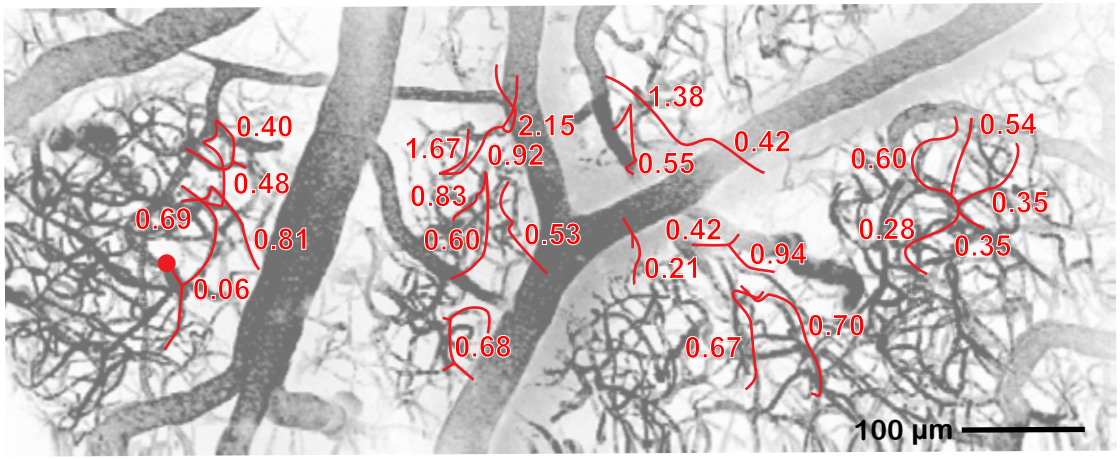


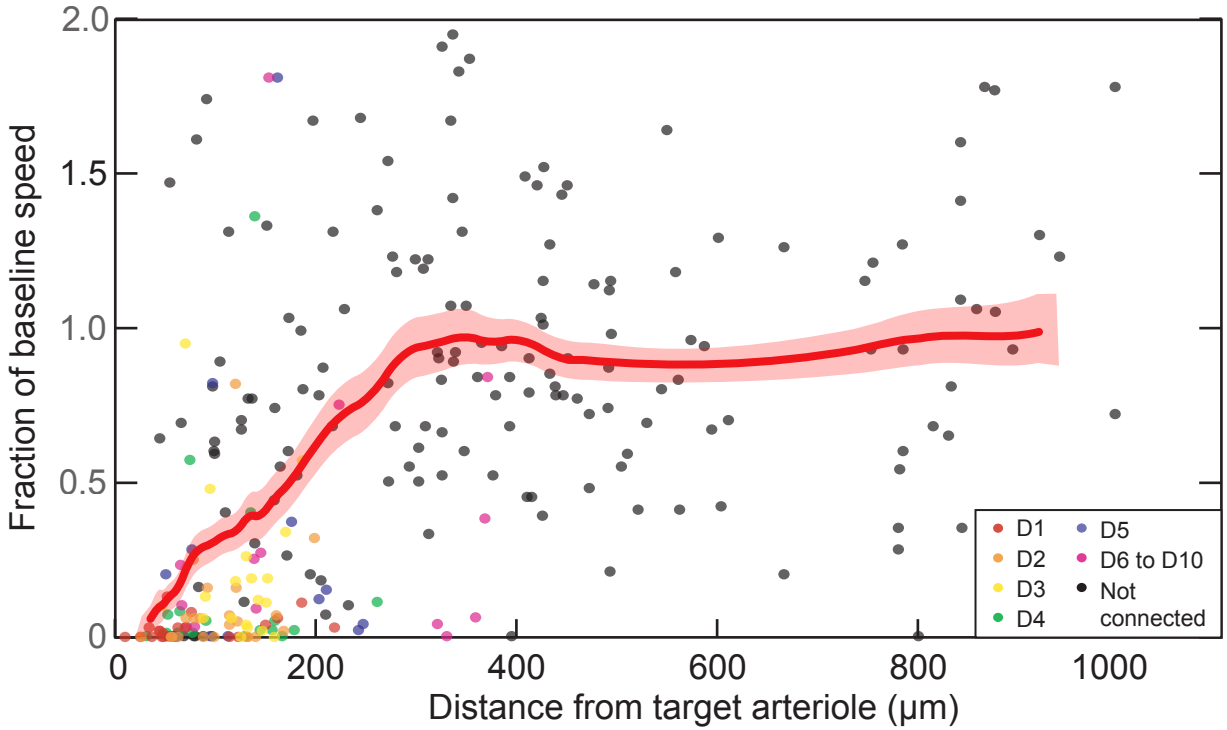


- Penetrating arteriole

Nishimura, Schaffer, Friedman, Lyden and Kleinfeld (PNAS 2006)

Occlusion of a penetrating arteriole retards flow within a 400 to 600 µm diameter “cylinder”





Nishimura, Schaffer, Friedman, Lyden and Kleinfeld (PNAS 2007)

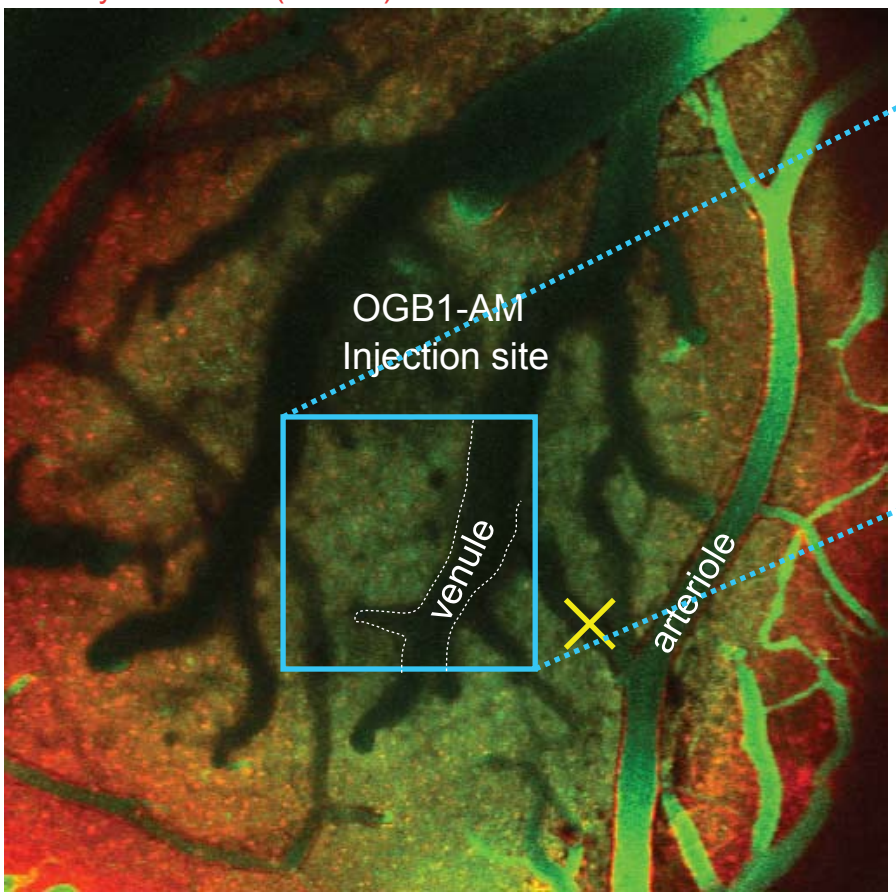
Lesson: Penetrating arterioles are a bottleneck in the supply of blood to the subsurface microvasculature.

Question: What is the chronic consequence of blockage of a single penetrating arteriole?

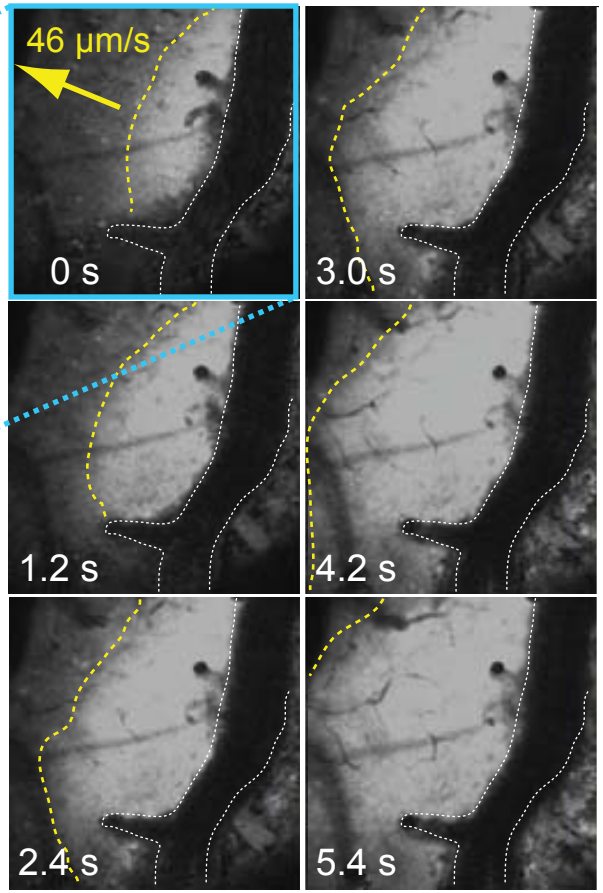
Blockage of a single penetrating arteriole leads to waves of cortical spreading depression

Pre-occlusion

Cell calcium indicator (OGB1-AM or FITC)
Astrocytes marker (SR101)

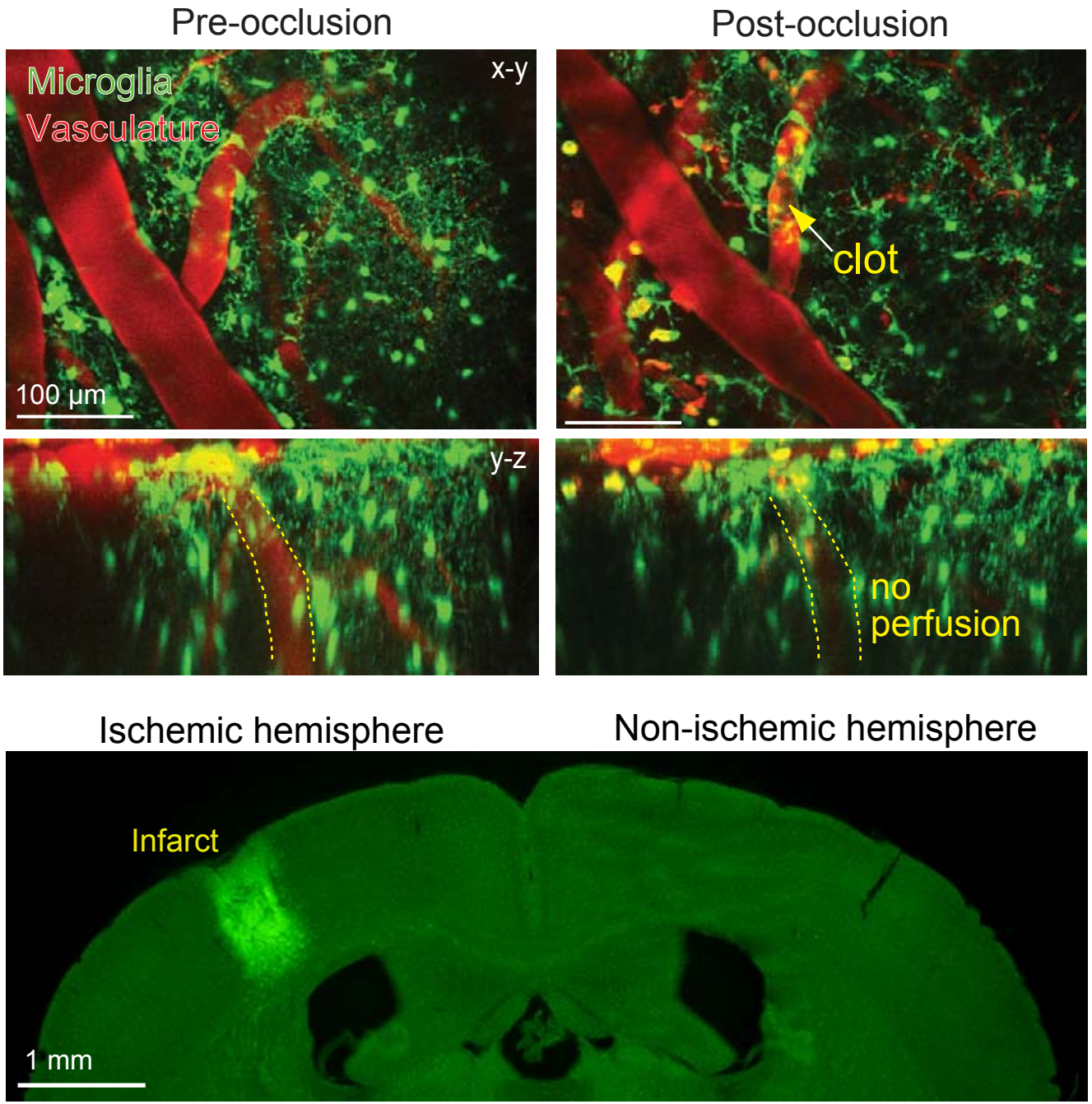


Post-occlusion



Shih, Tsai, Driscoll, Blinder, Drew, Friedman, Lyden & Kleinfeld (SfN abstract 2010)

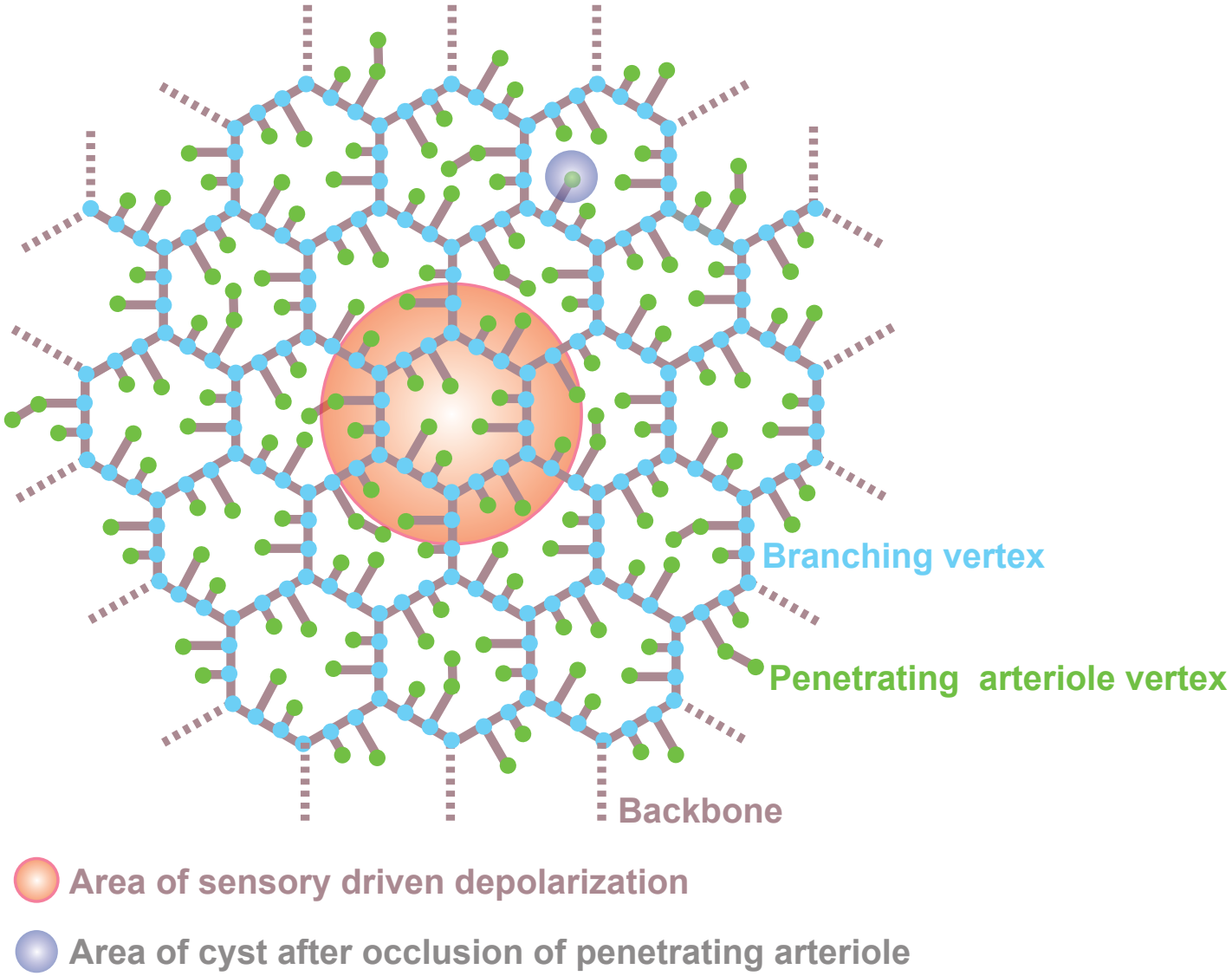
Microglial invasion after occlusion of a single penetrating arteriole



Drew, Shih, Driscoll, Knutsen, Blinder, Akassoglou, Davalos, Tsai & Kleinfeld (Nat Meth 2010)

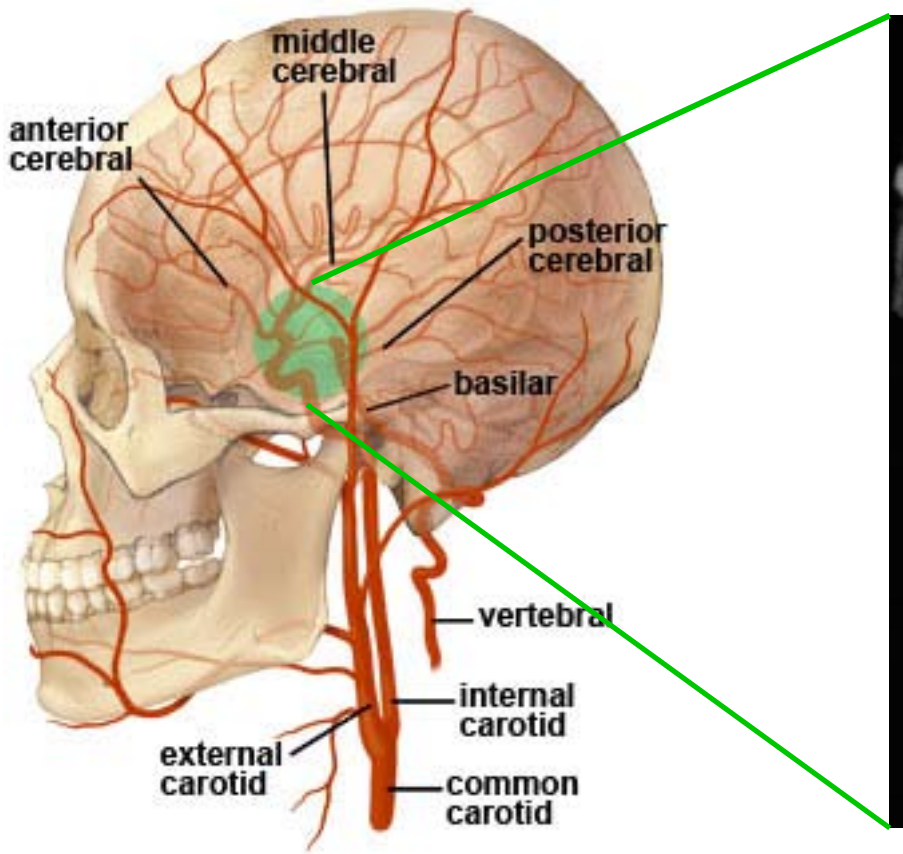
Lesson: Blockage of a single penetrating arteriole leads to cell death throughout a region of cortex.

Summary of idealized topology and relative scales of the pial vasculature



Blinder*, Shih*, Rafie and Kleinfeld (PNAS 2010)

Surface loops add redundancy that is reminiscent of Circle of Willis



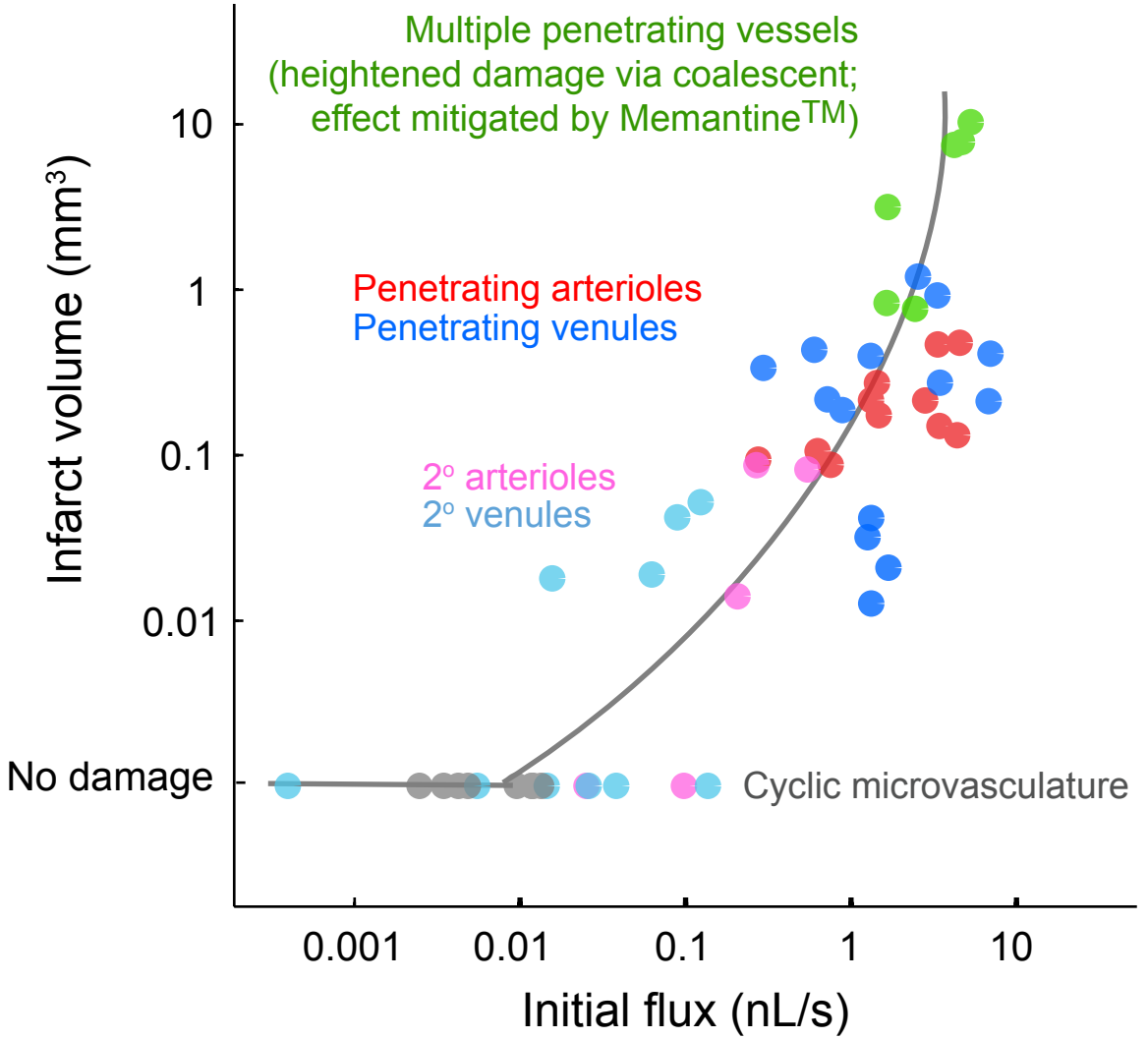
Thomas Willis (*Cerebri anatome* 1664)

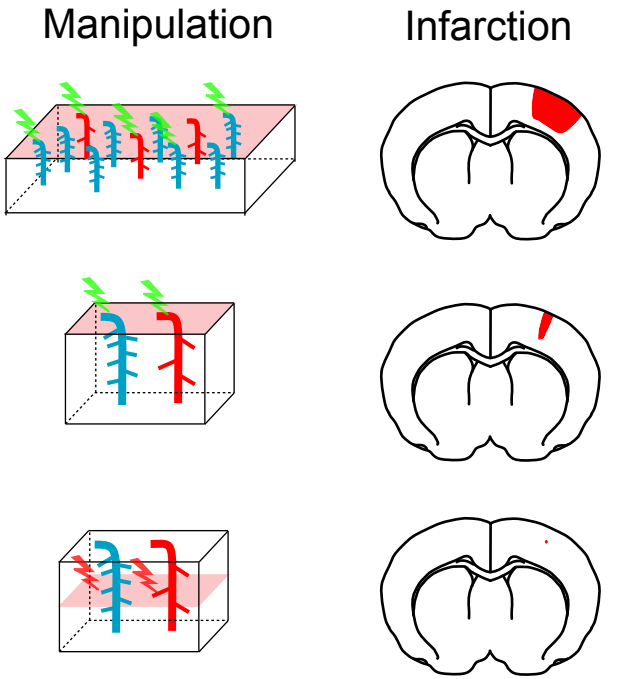
These results open questions in biomedicine *and* biofluidics

Biomedicine: Are cortical micro-infarctions, seen in vascular dementia, a consequence of occluded penetrating vessels?

Biofluidics: Does each penetrating arteriole have an exclusive territory or does the subsurface microvasculature form a continuous network?

Hierarchy of microinfarcts from occlusions to 1-D penetrating vessels or their immediate downstream branches





Schaffer, Friedman, Nishimura, Schroeder, Tsai, Ebner, Lyden & Kleinfeld (PLoS Biology 2006);
Shih, Friedman, Drew, Blinder, Lyden & Kleinfeld (JCBFM 2009); Shih, Tsai, Driscoll, Blinder, Drew, Friedman, Lyden & Kleinfeld (SfN abstract 2010)

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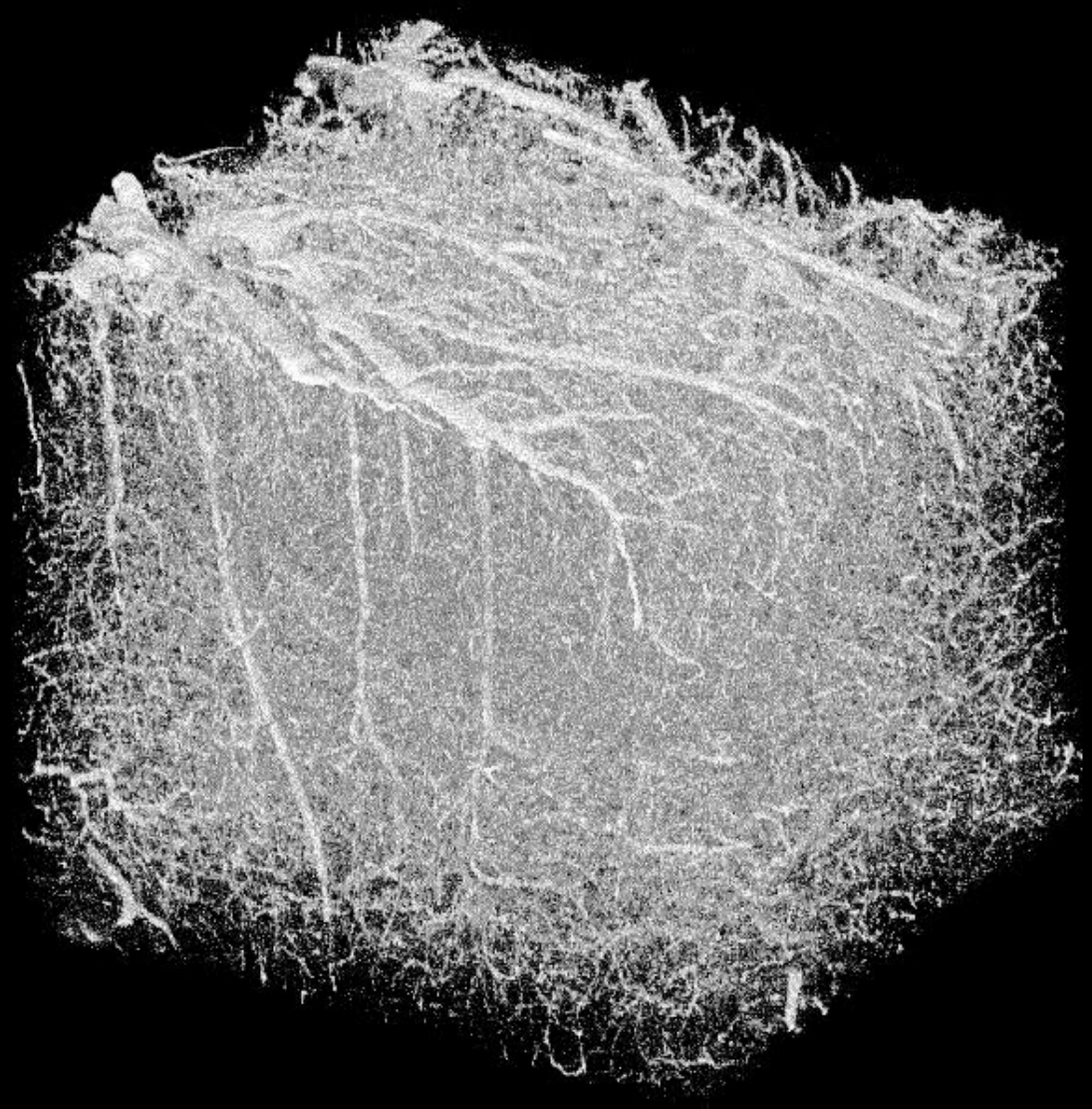
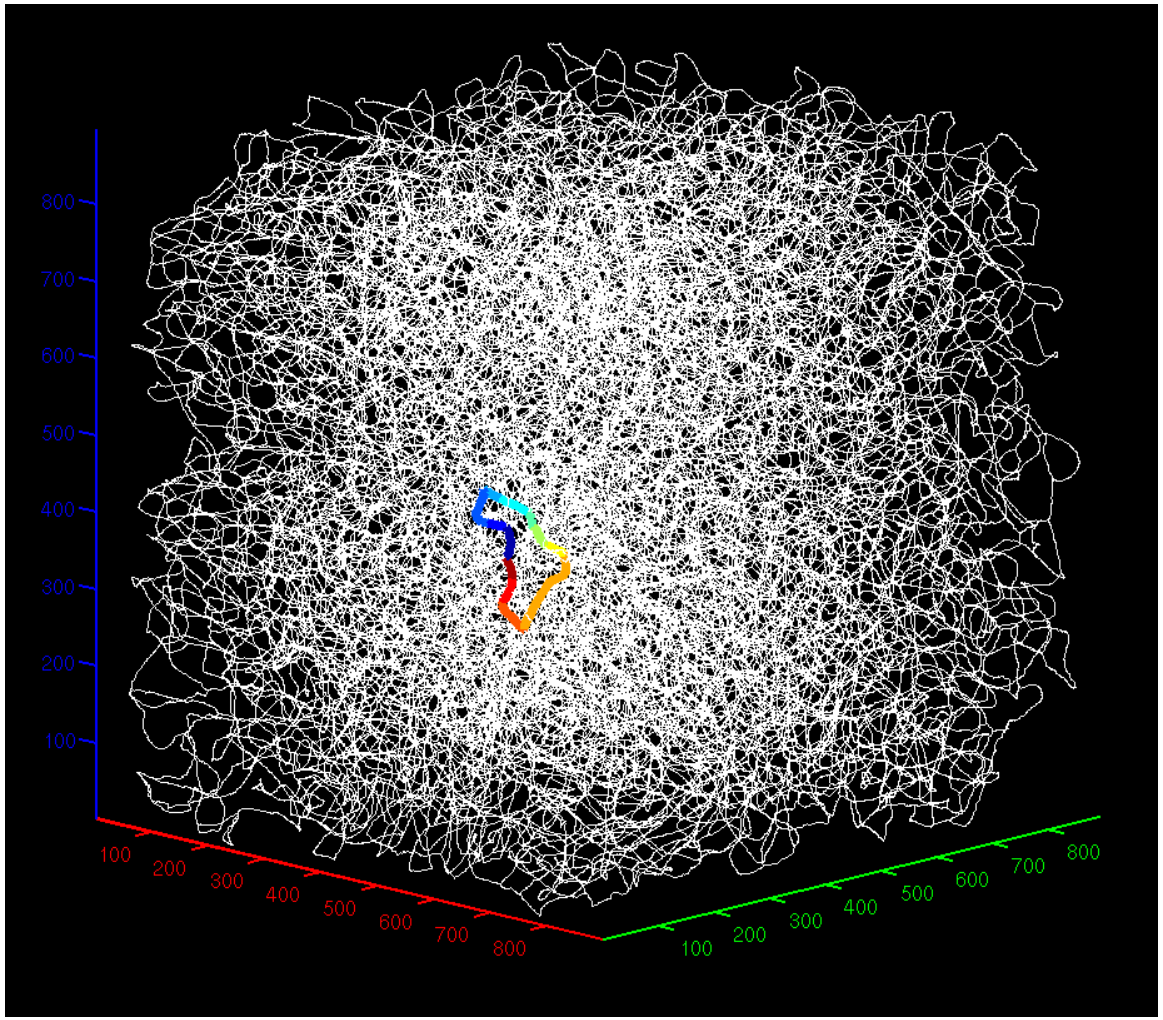
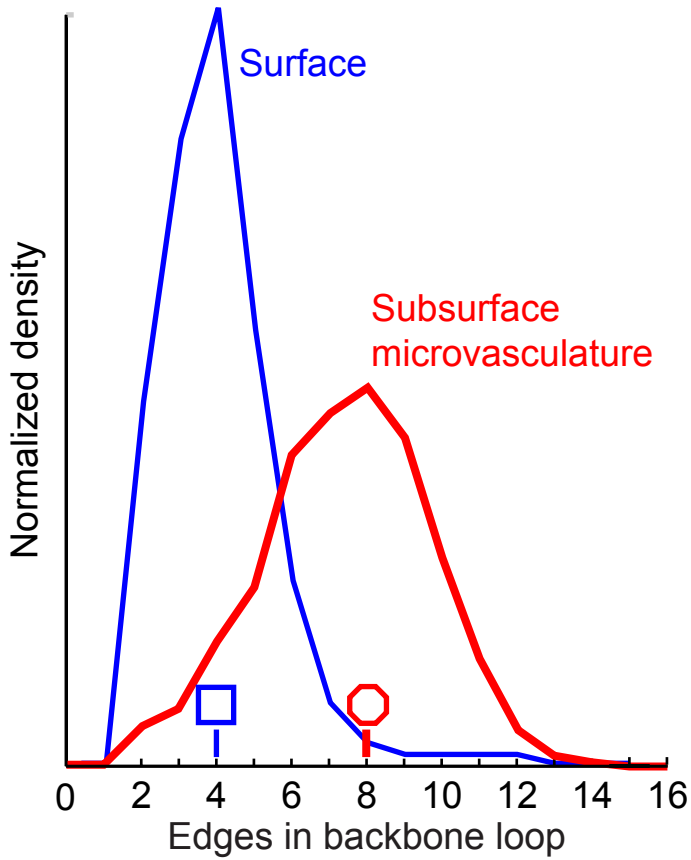
Highlights of the rodent angiotome: A work in progress



Adapted from Tsai, Kaufhold, Blinder, Friedman, Dew, Karten, Lyden & Kleinfeld (2009) and Blinder*, Tsai*, Kaufhold & Kleinfeld (in preparation)

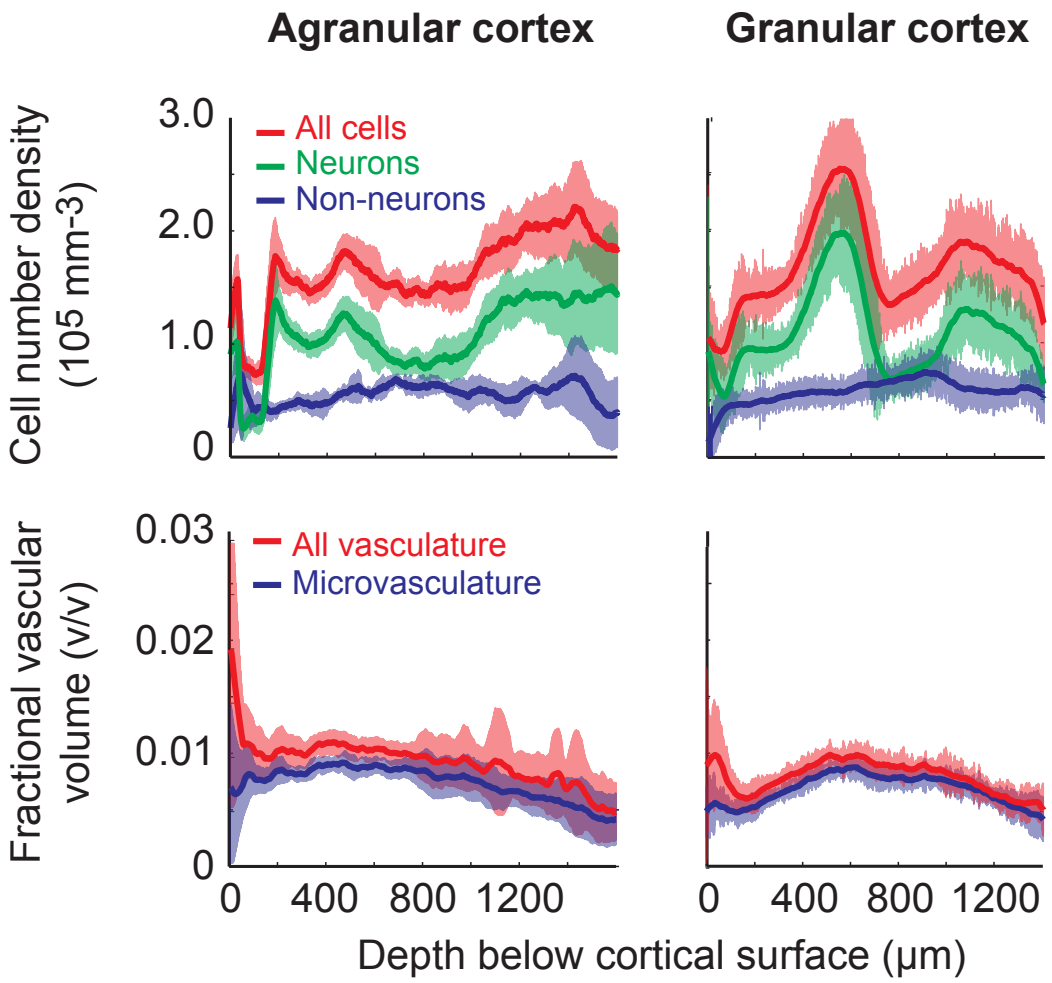
Subsurface microvessels form a highly interconnected network

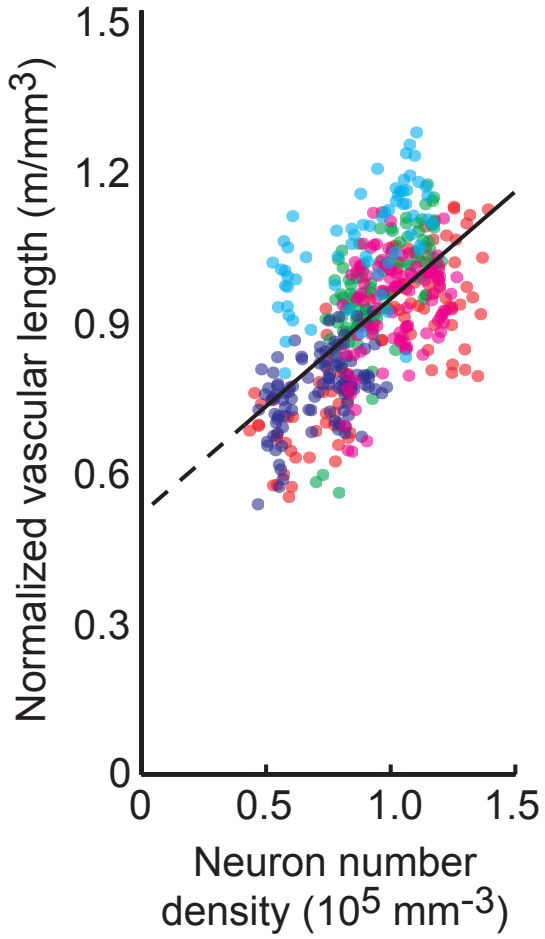




BlinderP *, Tsai PS*, Kaufhold JK & Kleinfeld D (unpublished)

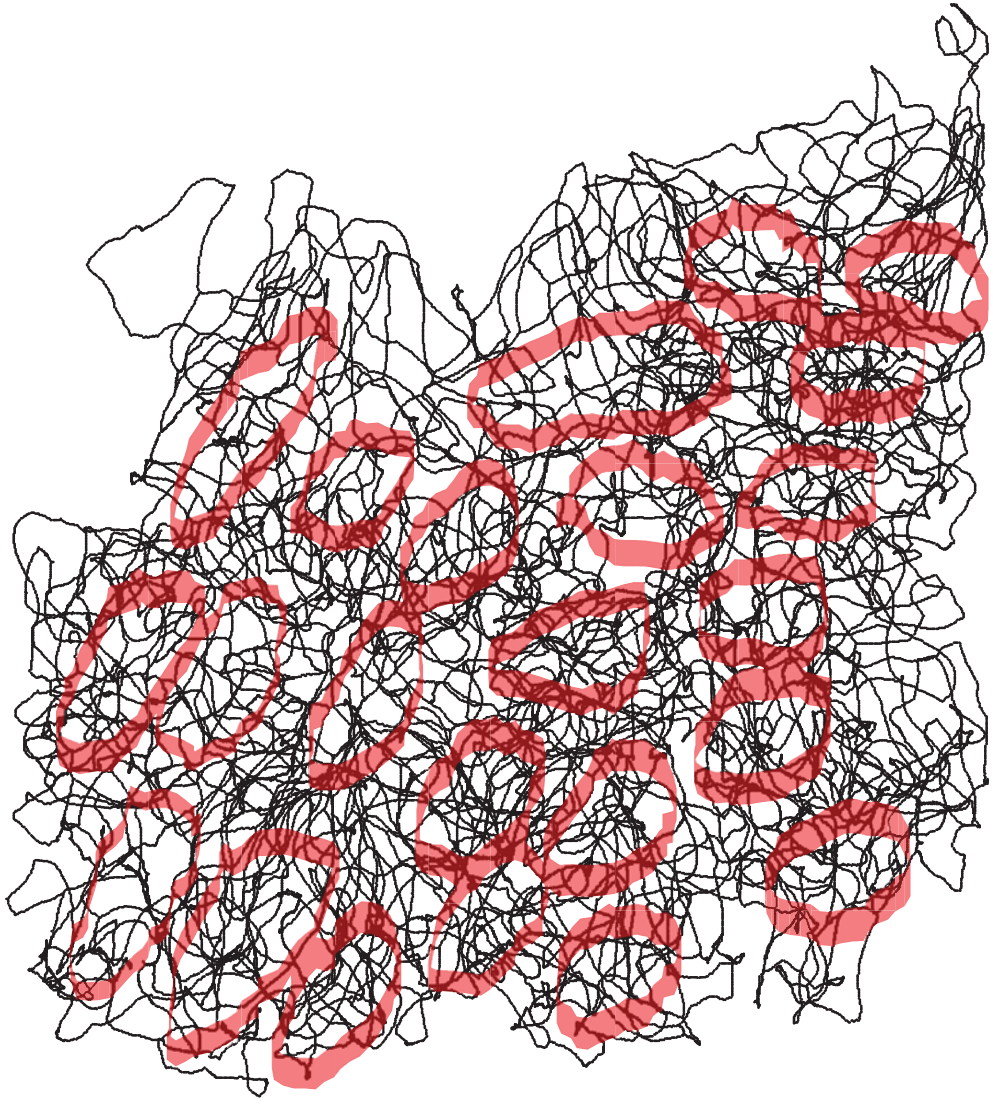
Statistics of vessels and cells in mouse neocortex from a complete inventory across 1 - 10 mm^3 slabs





Tsai, Kaufhold, Blinder, Friedman, Drew, Karten, Lyden & Kleinfeld (Journal of Neuroscience 2009)

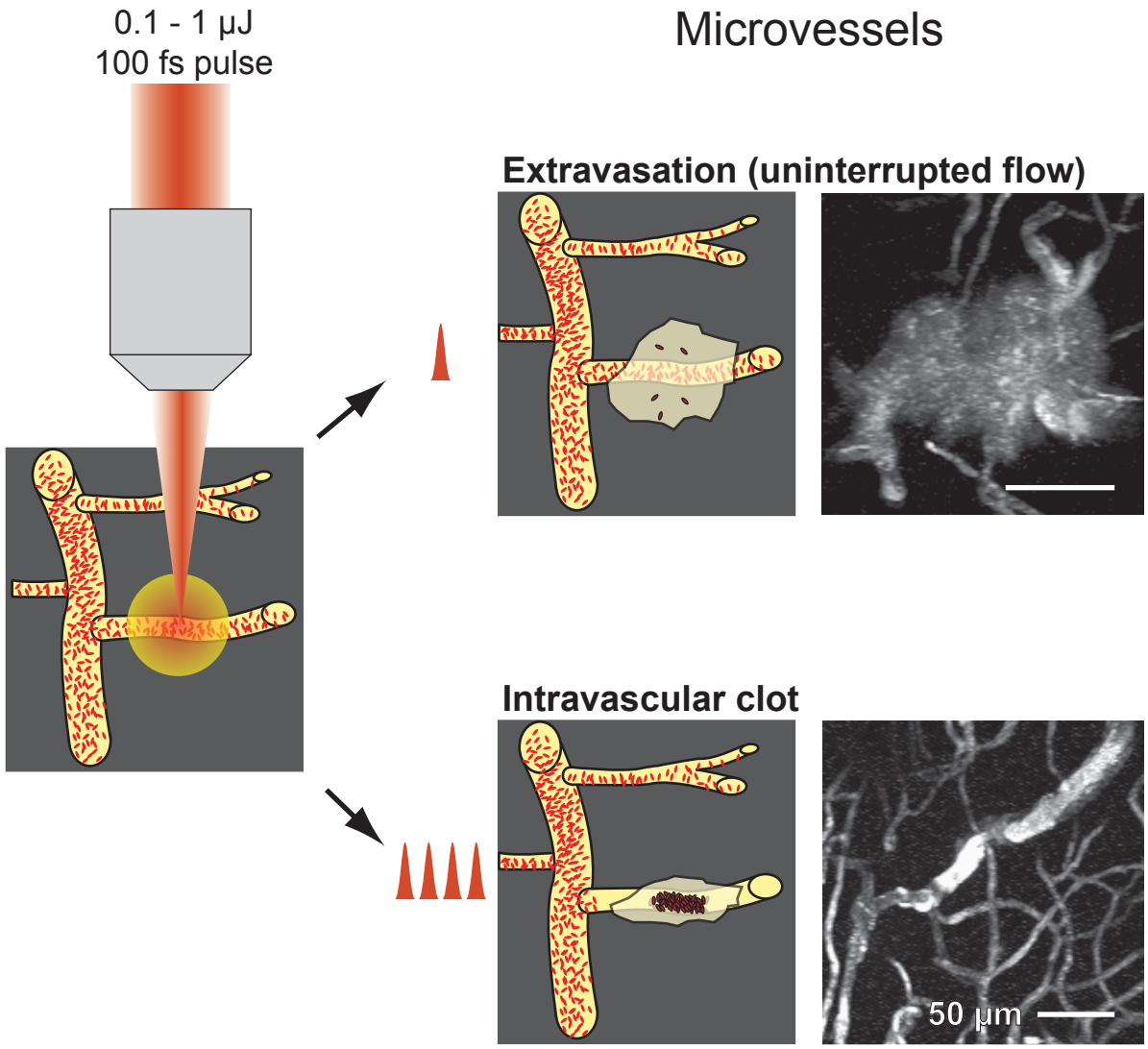
The set of shortest cycles in layer IV form a single connected graph



Blinder P*, Tsai PS*, Kaufhold J. and Kleinfeld D. (unpublished)

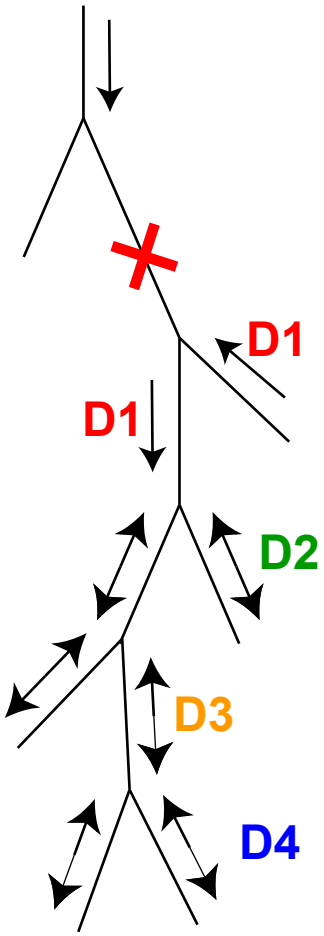
Question: What is the consequence of blockage to a single microvessel, *i.e.*, do subsurface loops protect local flow?

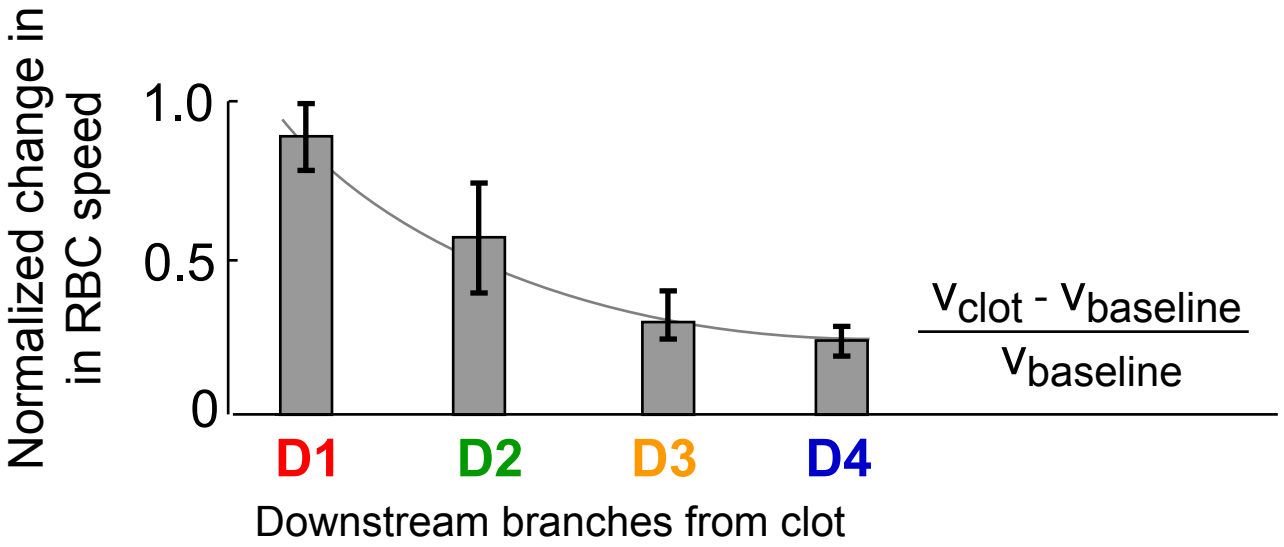
Targeted disruption of subsurface microvessels by amplified laser pulses



Nishimura, Schaffer, Friedman, Tsai, Lyden & Kleinfeld (Nat Meth 2006)
Tsai, Blinder, Migliori, Neev, Jin, Squier & Kleinfeld (Cur Opin Biotech 2009)

Targeted occlusion to a subsurface microvessel blocks flow only in nearest, but not nearby, downstream branches





Consistent with long microvascular loops

Nishimura, Schaffer, Friedman, Tsai, Lyden and Kleinfeld (Nat Meth 2006)

Lesson: Microvessels form closed loops that span multiple cortical columns – no evidence for exclusive territories.

Question: Why is flow spatially restricted even though we have complete lateral connectivity?

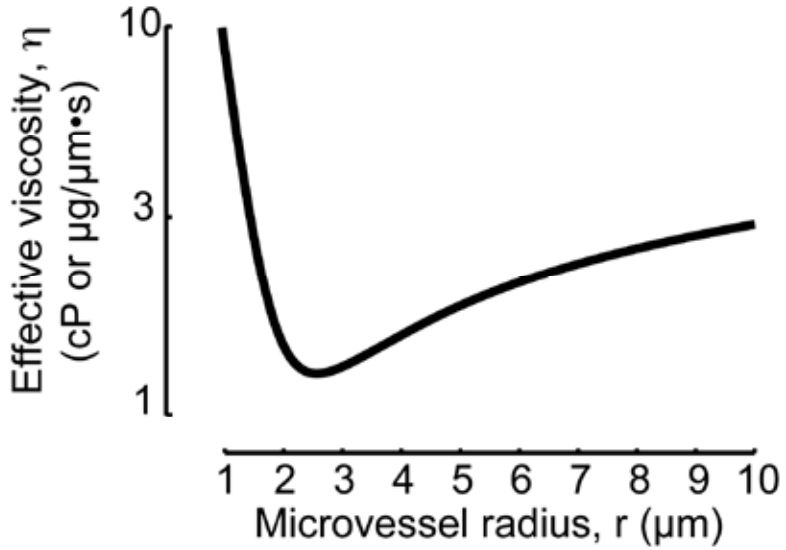
Flow calculated between pairs of vertices using Kirchhoff's Law

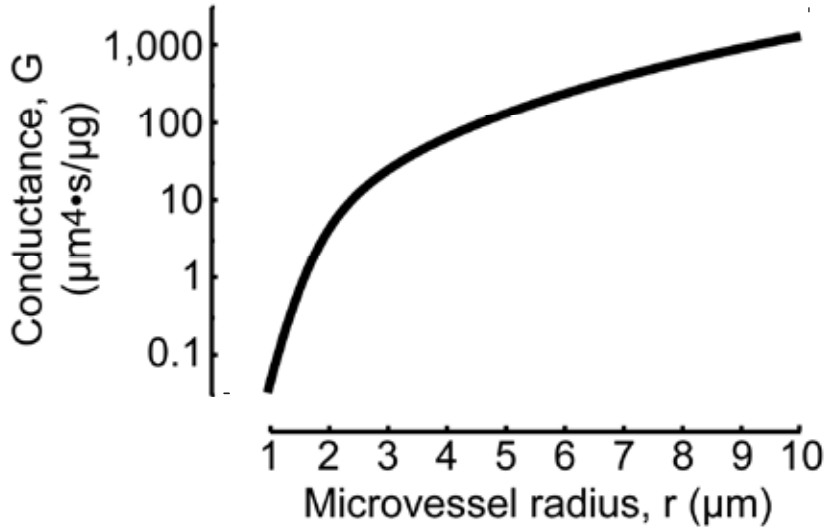
Volume flux = [Conductance] (Pressure at vertex)

$$\mathbf{F = G\,P}$$

For each pair of vertices (n,m), the measured vessel length l_{nm} and radius r_{nm} form the elements of **G**

$$G_{nm} = \frac{\pi}{8}\,\frac{r_{nm}^4}{\eta\left(r_{nm}\right)}\,\frac{1}{l_{nm}} \qquad \text{with} \qquad G_{nn} = -\sum_{m\neq n}^{\text{all vessels}} G_{nm}$$





Pries & Secomb (American Journal of Physiology HCP 2005)

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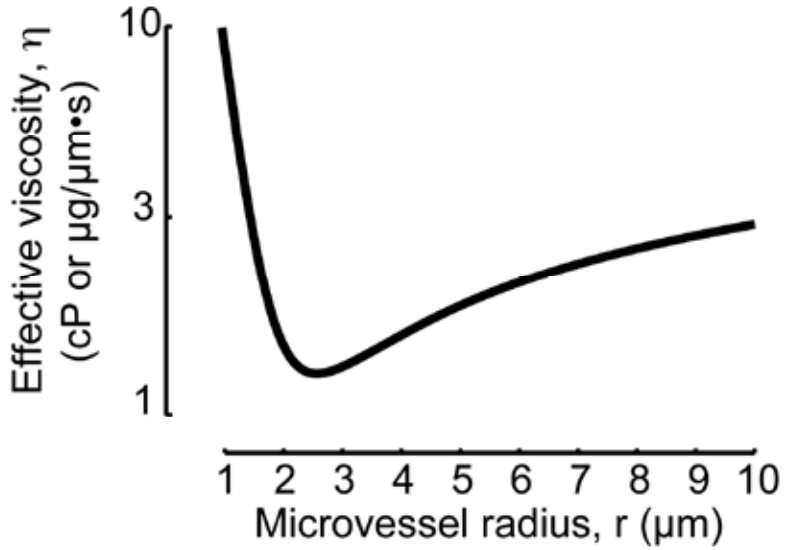
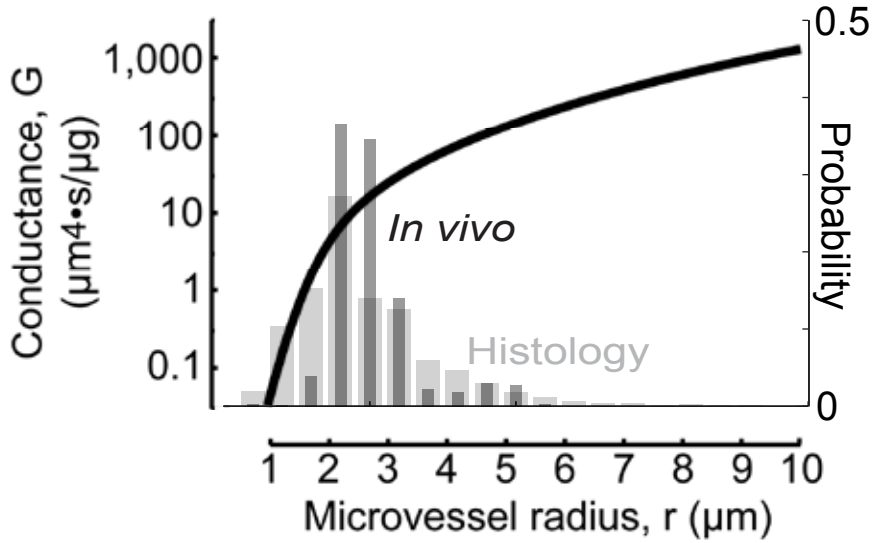
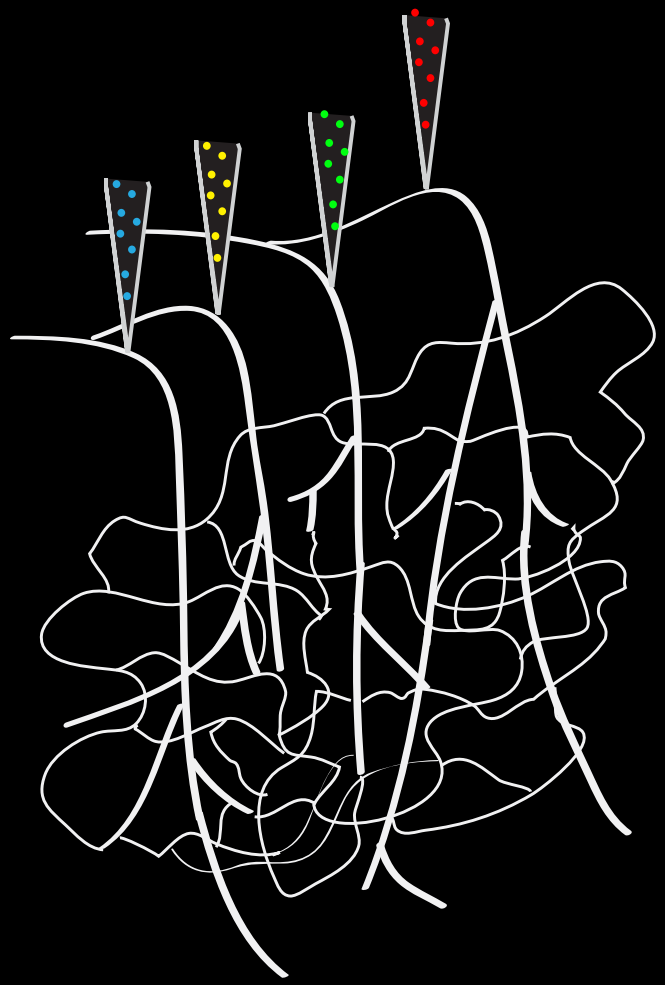




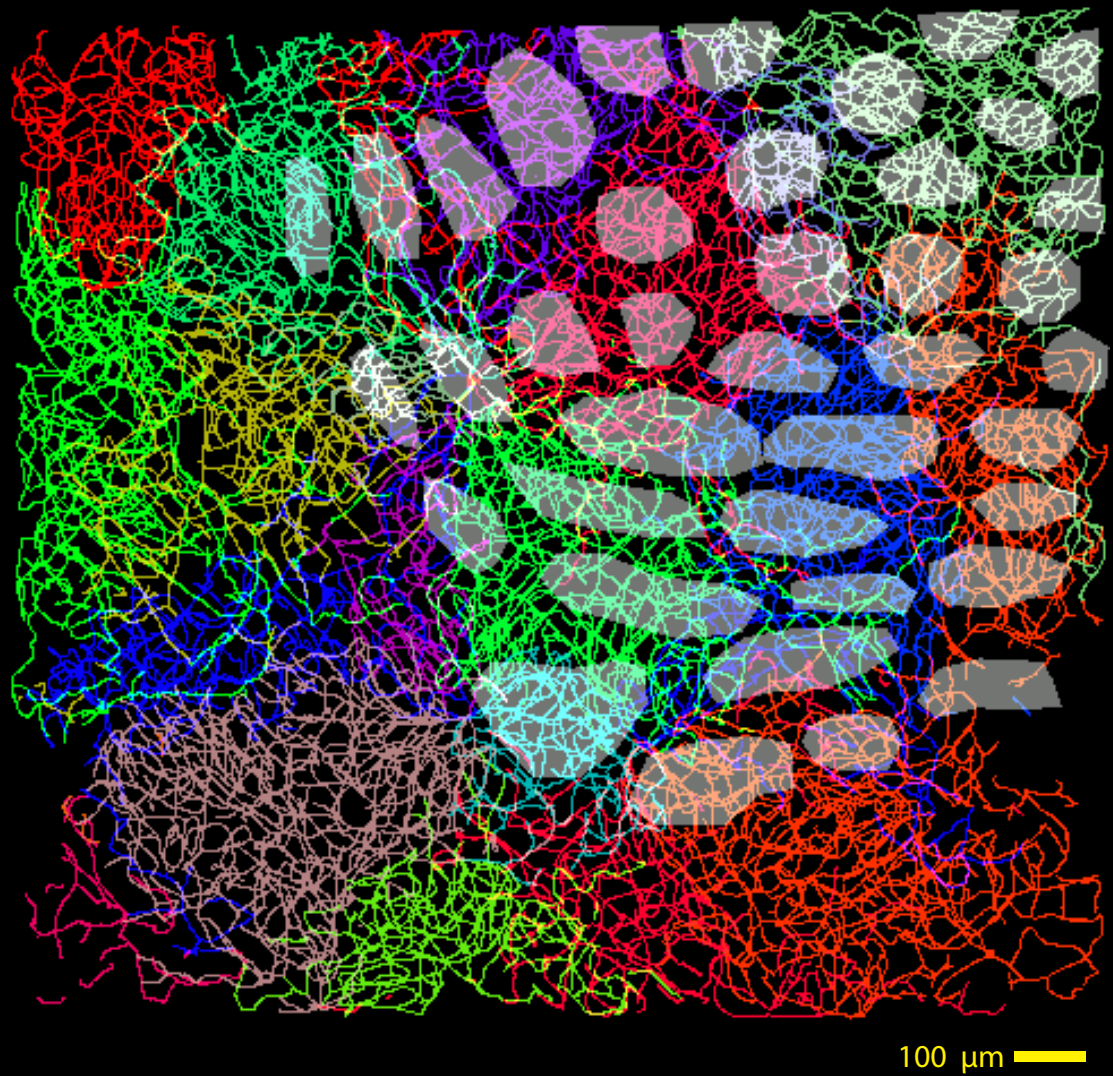
Pries & Secomb (American Journal of Physiology HCP 2005)

Tsai, Kaufhold, Blinder, Friedman, Drew, Karten, Lyden & Kleinfeld (J Neuroscience 2009)

Numerical analysis of “dye” injection to determine perfusion domains of individual penetrating arterioles

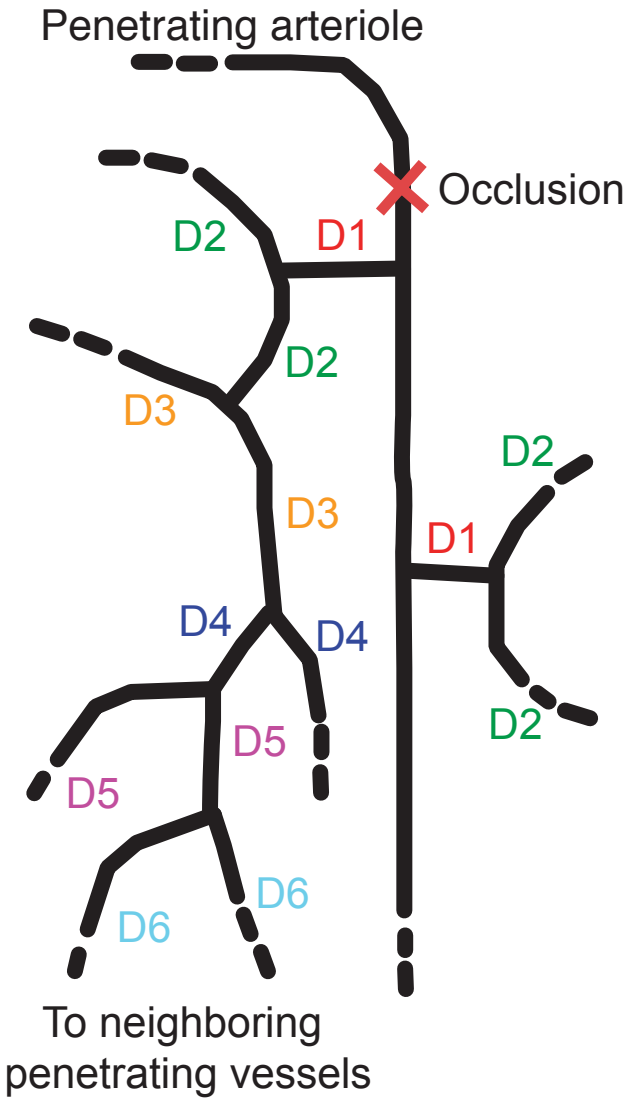


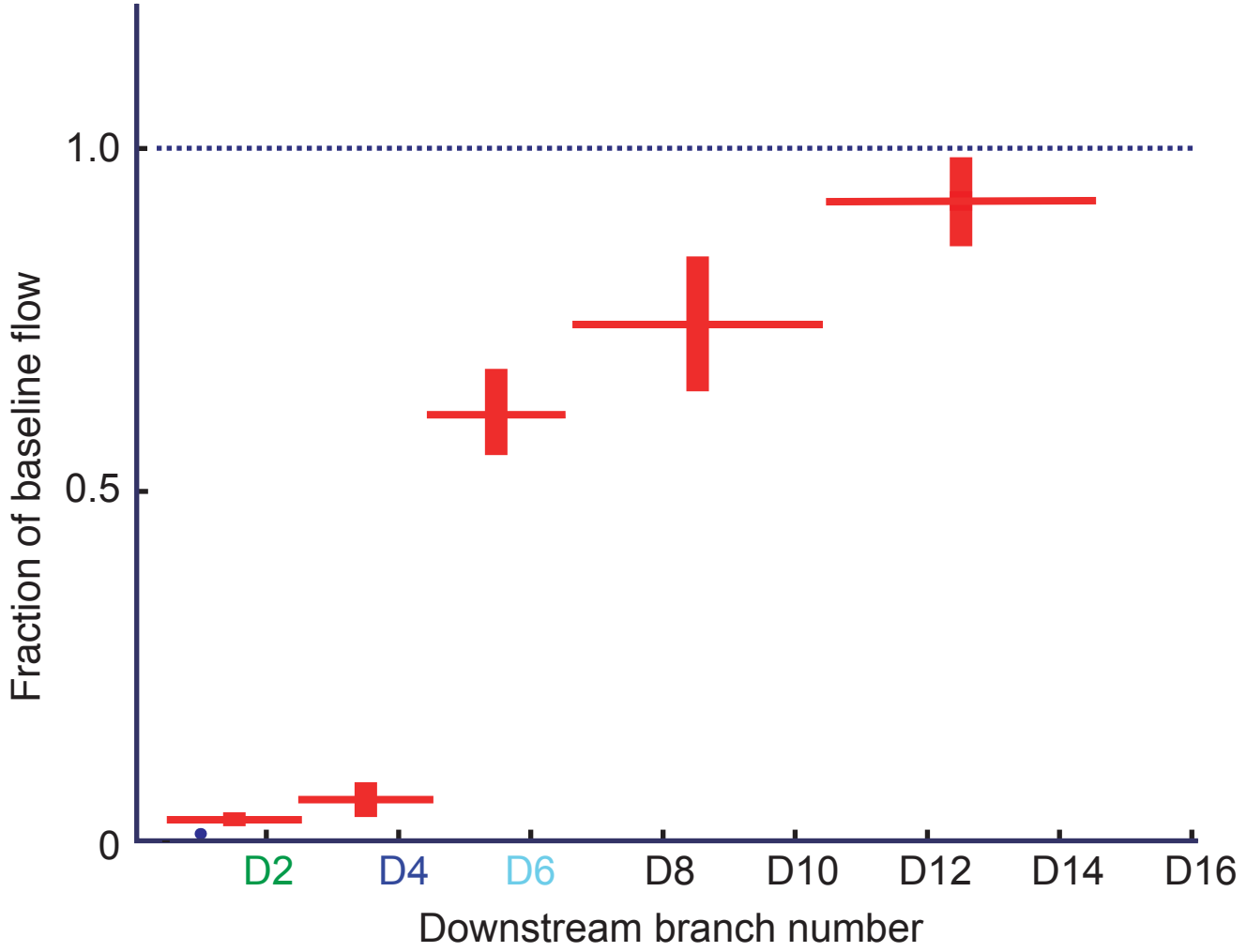
L4 (sensory input layer)



Blinder*, Tsai *, Kaufhold & Kleinfeld (in progress)

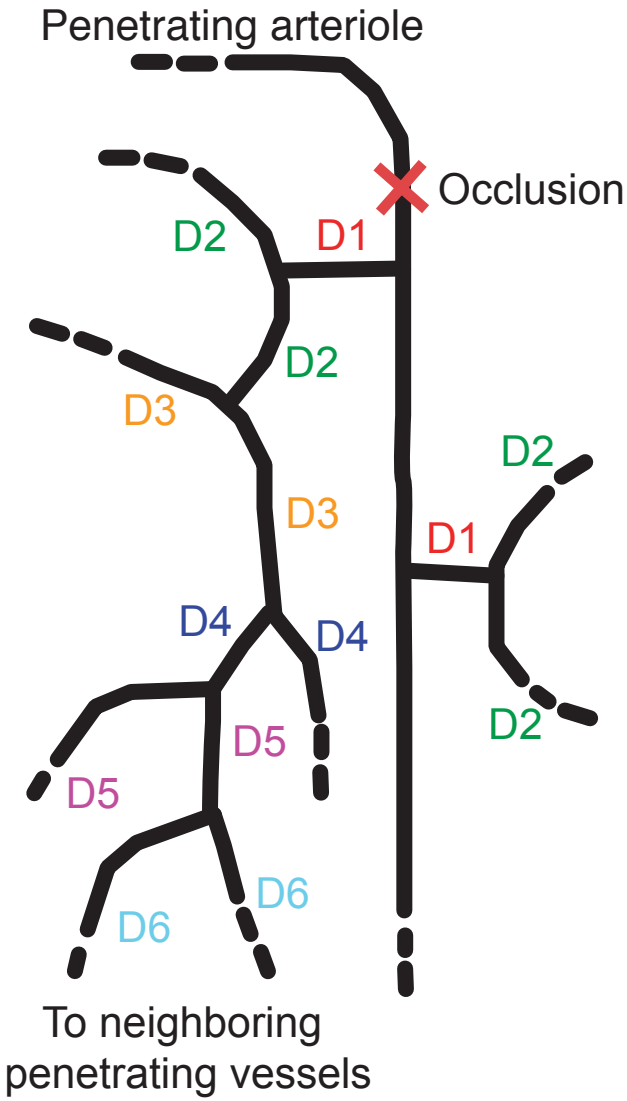
Passive flow models the effect of an occlusion to a penetrating arteriole

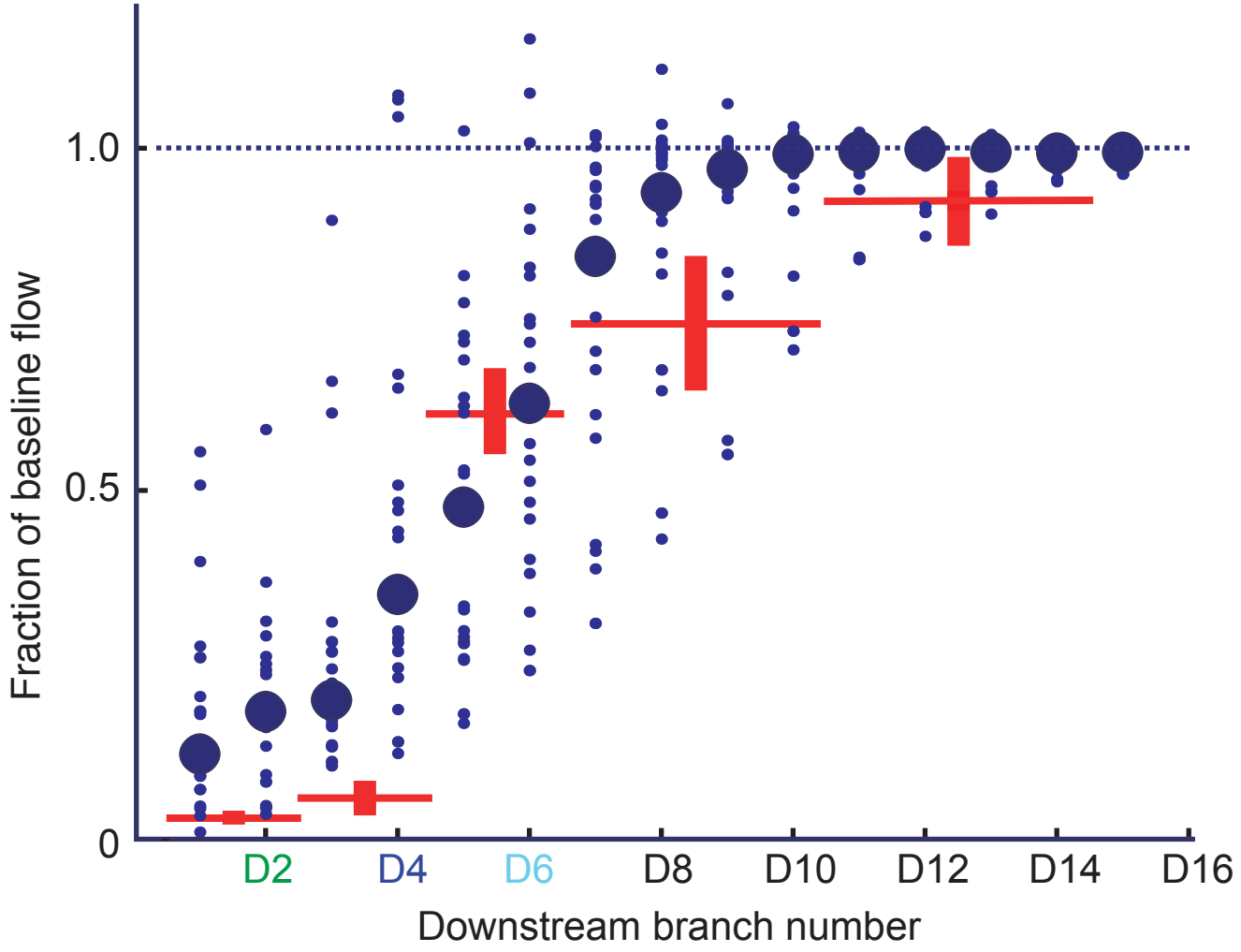




In vivo rat data of Nishimura, Rosidi, Iadecola & Schaffer (JCBFM 2010)

Passive flow models the effect of an occlusion to a penetrating arteriole





In vivo rat data of Nishimura, Rosidi, Iadecola & Schaffer (JCBFM 2010)

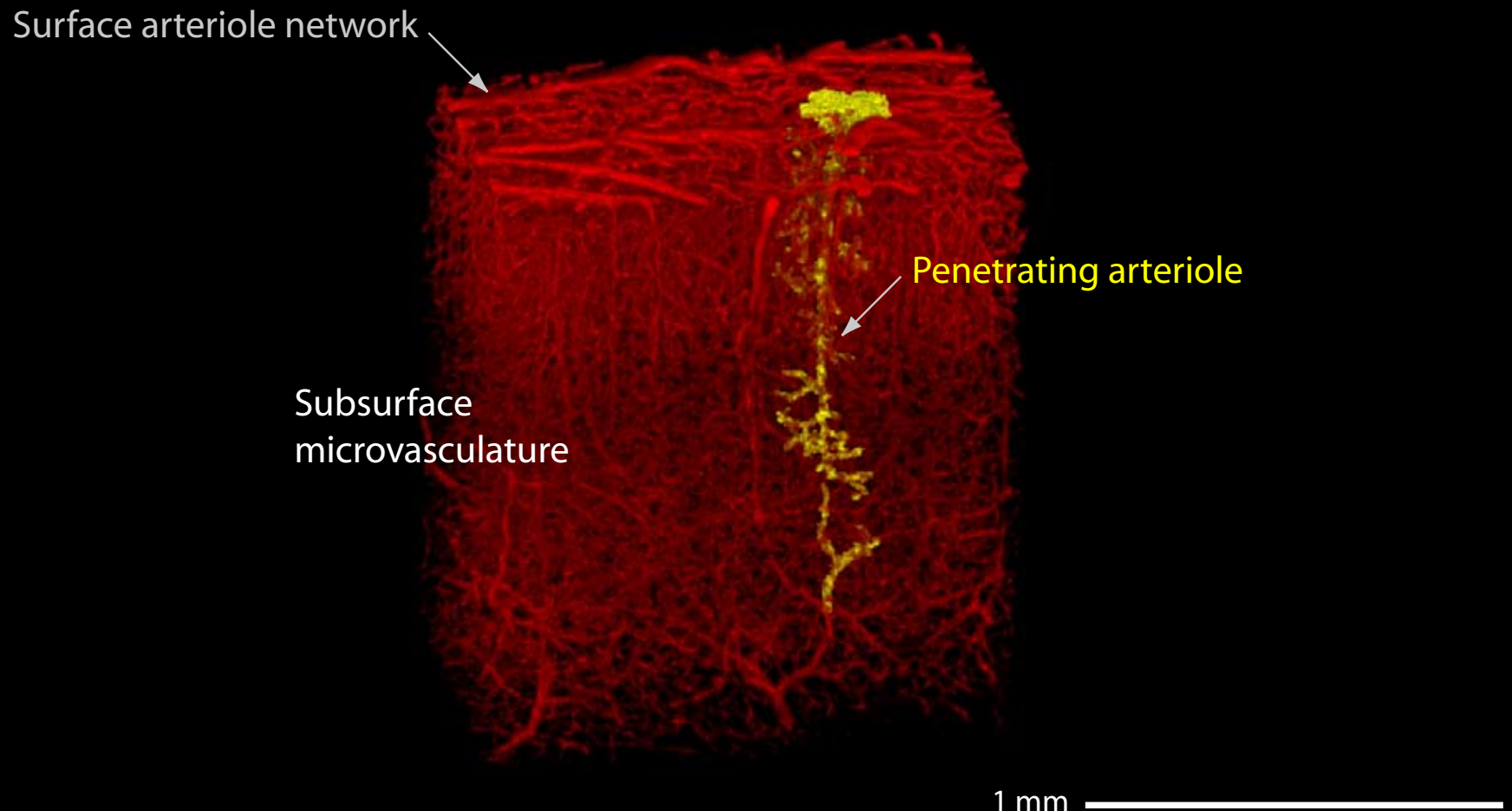
Flow analysis using mouse vascular reconstruction and Secomb's G(r, l).

Lesson: Lateral connectivity of the subsurface vasculature does not guarantee the perfusion of brain tissue.

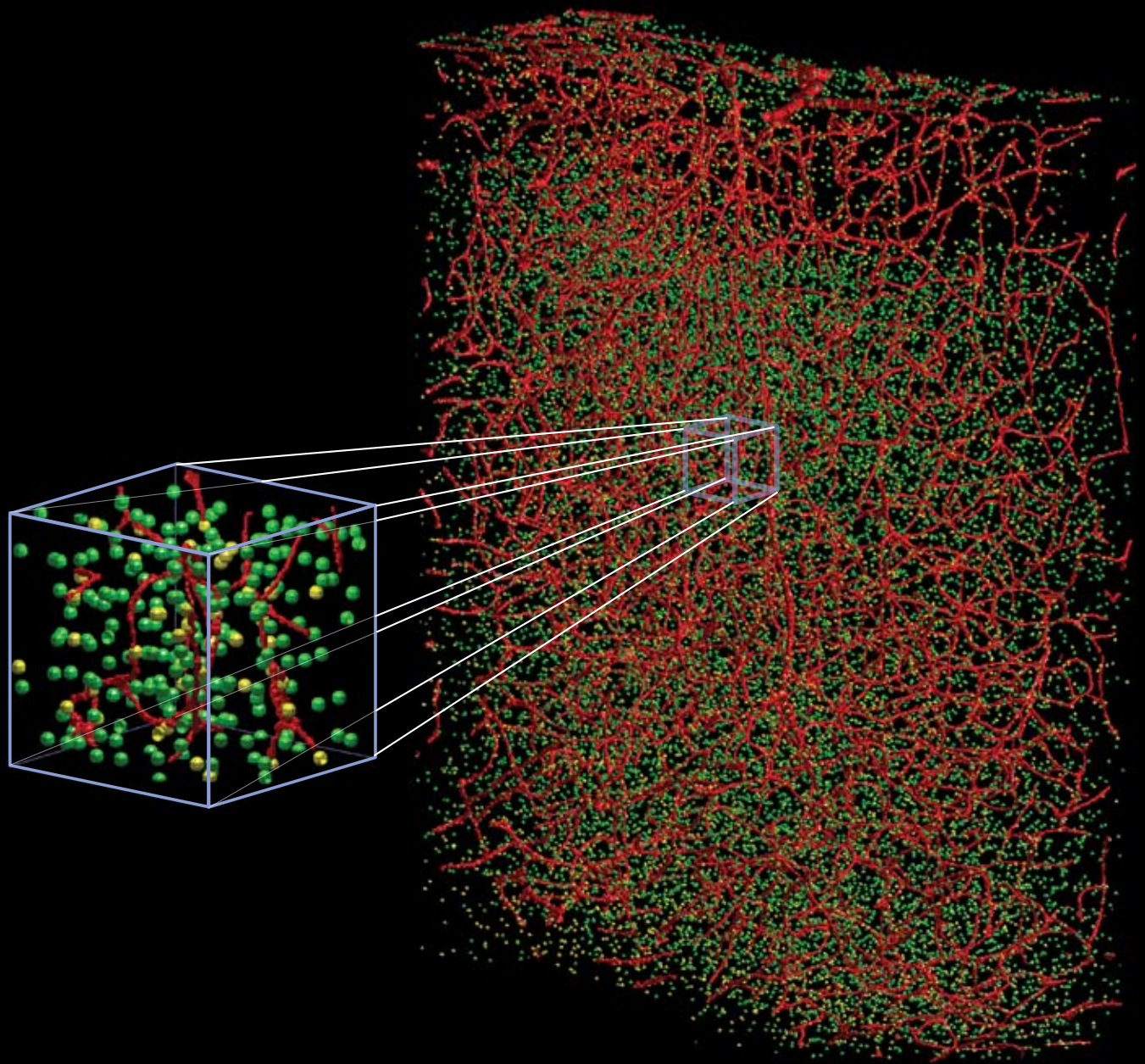
Summary on angioarchitecture

Redundancy: Surface vascular network as a 2-D distribution grid
Subsurface microvascular network as a 3-D grid

Fragility: Penetrating vessels as 1-D conduits from surface to microvessels
Subsurface microvascular dominated by isotropic resistance to flow



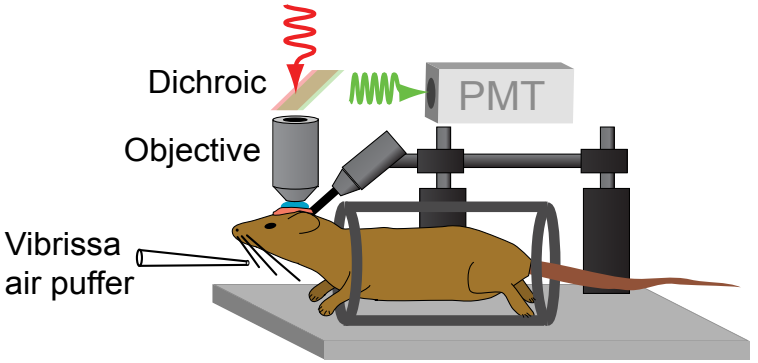
Next steps: Delimiting changes in blood flow by patterns of neuronal activity

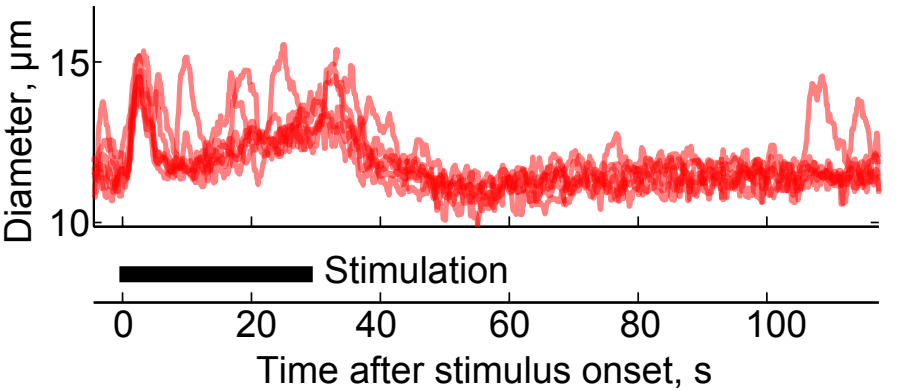


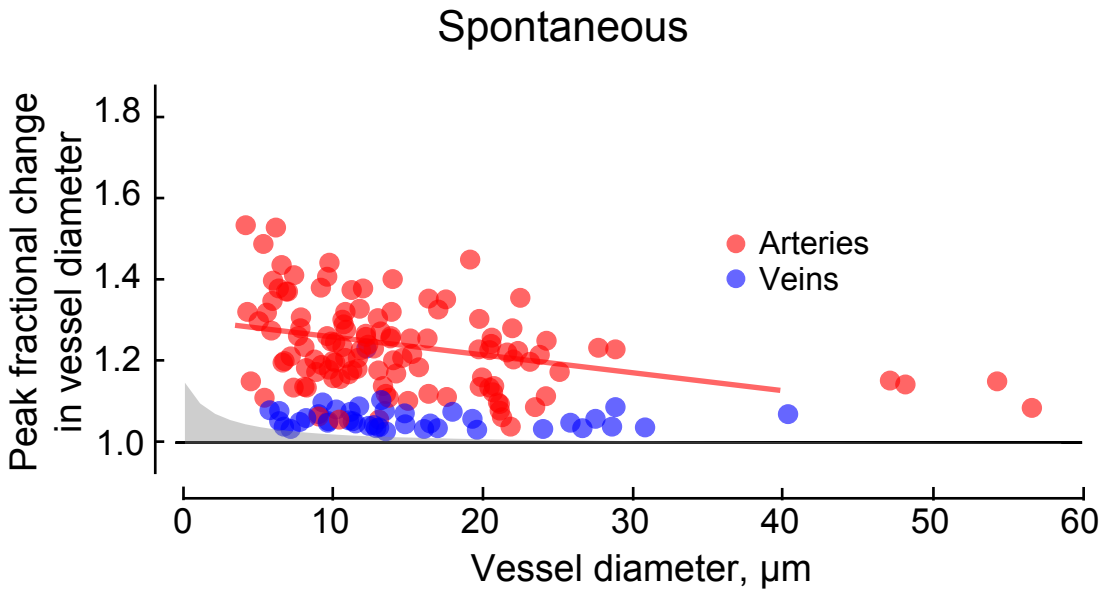
Tsai, Kaufhold, Blinder, Friedman, Drew, Karten, Lyden & Kleinfeld (J Neuroscience,2009)

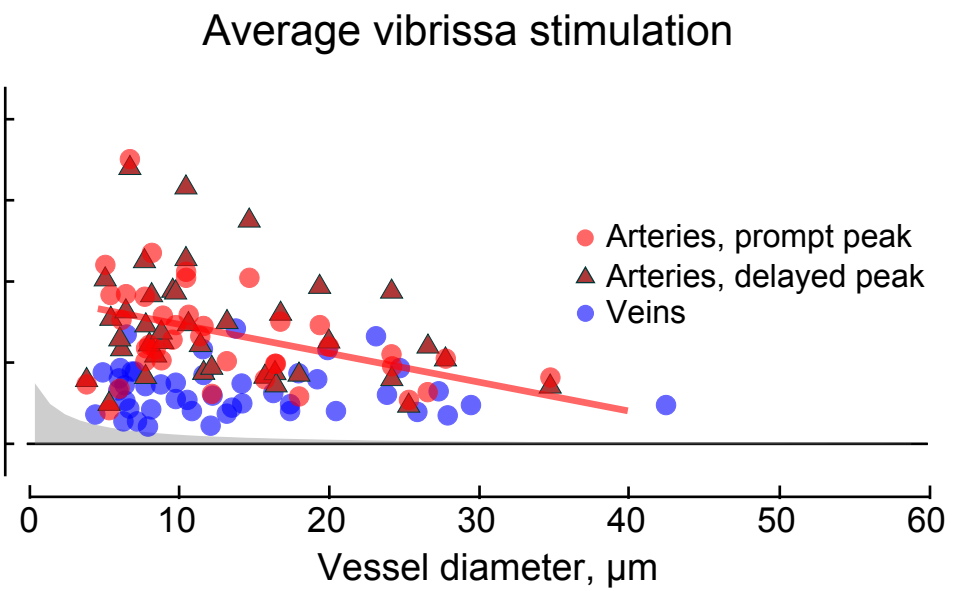
Question: How much does cortical blood flow fluctuate in the absence of sensory input?

Changes in arteriole diameter in response to stimulation are the same magnitude as the fluctuations in diameter (vasomotion)



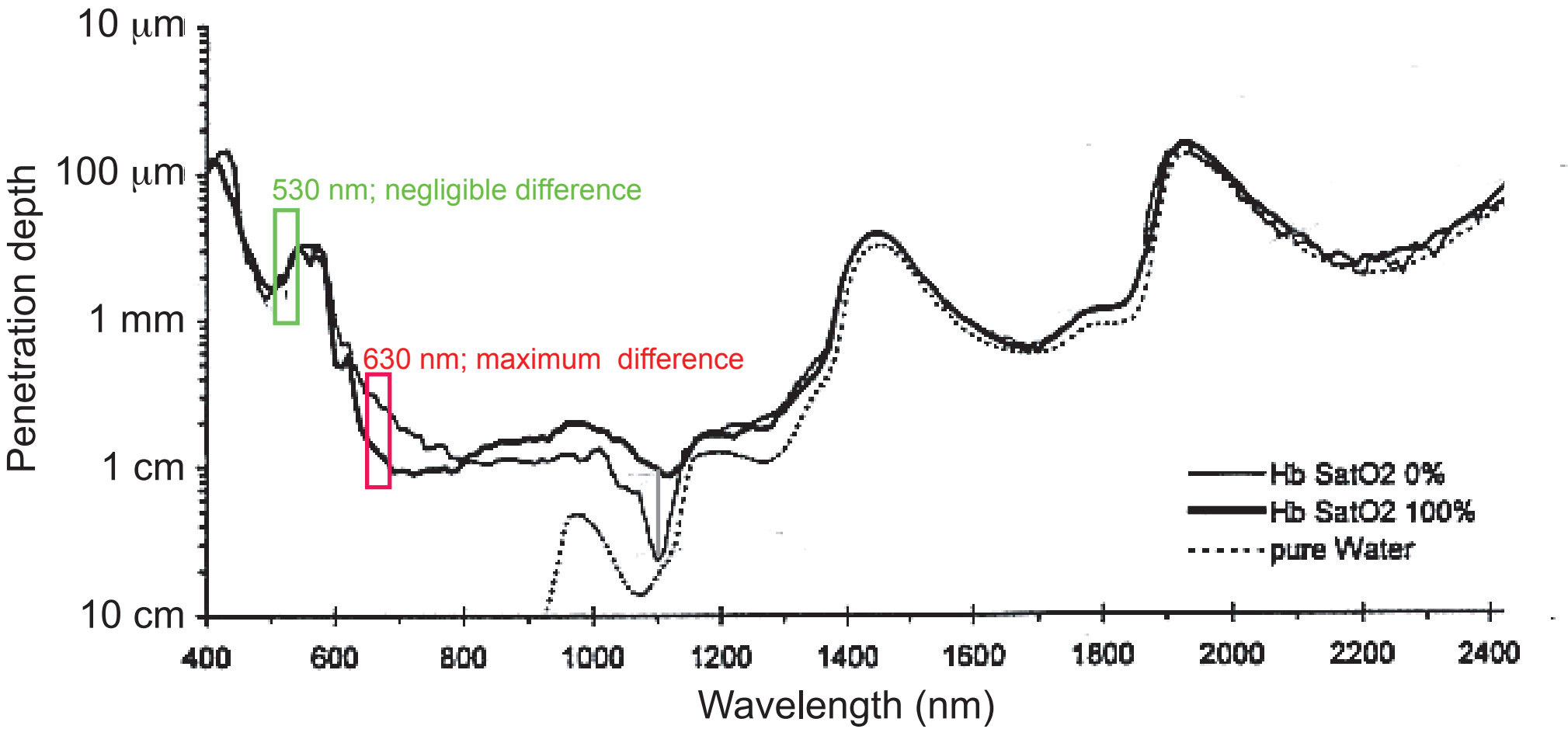




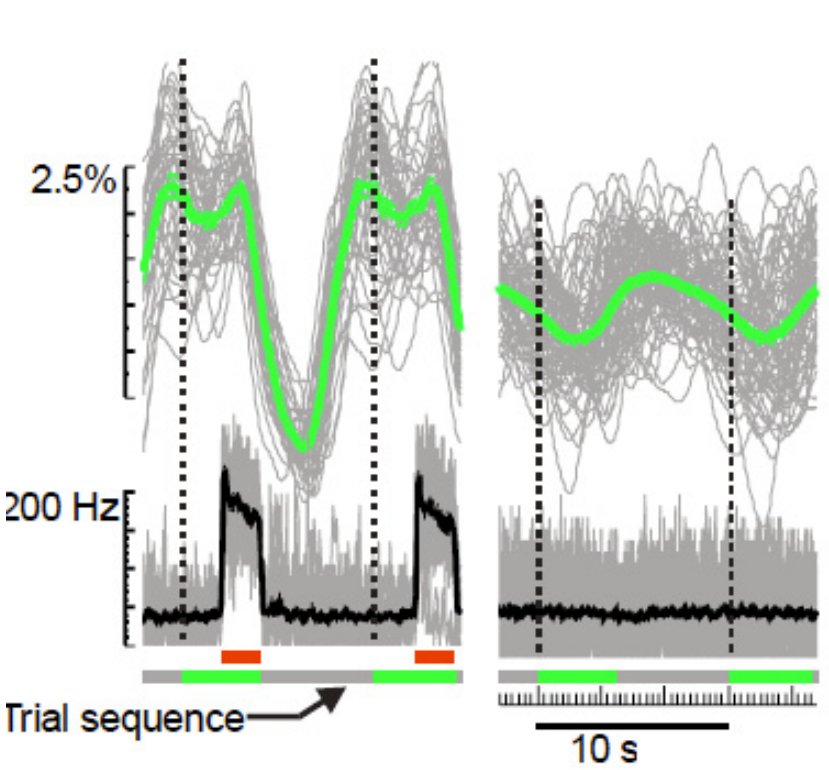


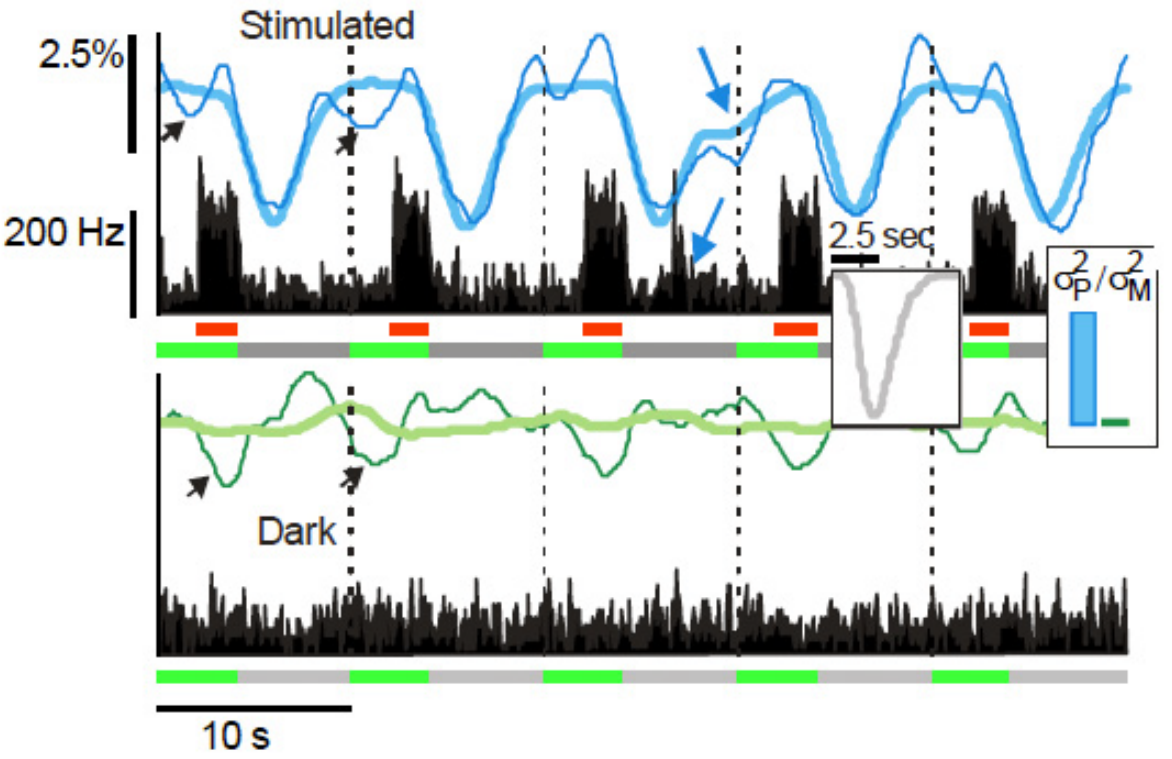
Drew, Shih & Kleinfeld (PNAS 2011)

Absorption by oxyhemeoglobin versus deoxyhemoglobin: Origin and BOLD fMRI and IOS signals



Changes in blood oxygenation synchronized to trial onset and not stimulation





Sirotin & Das (Nature 2009)

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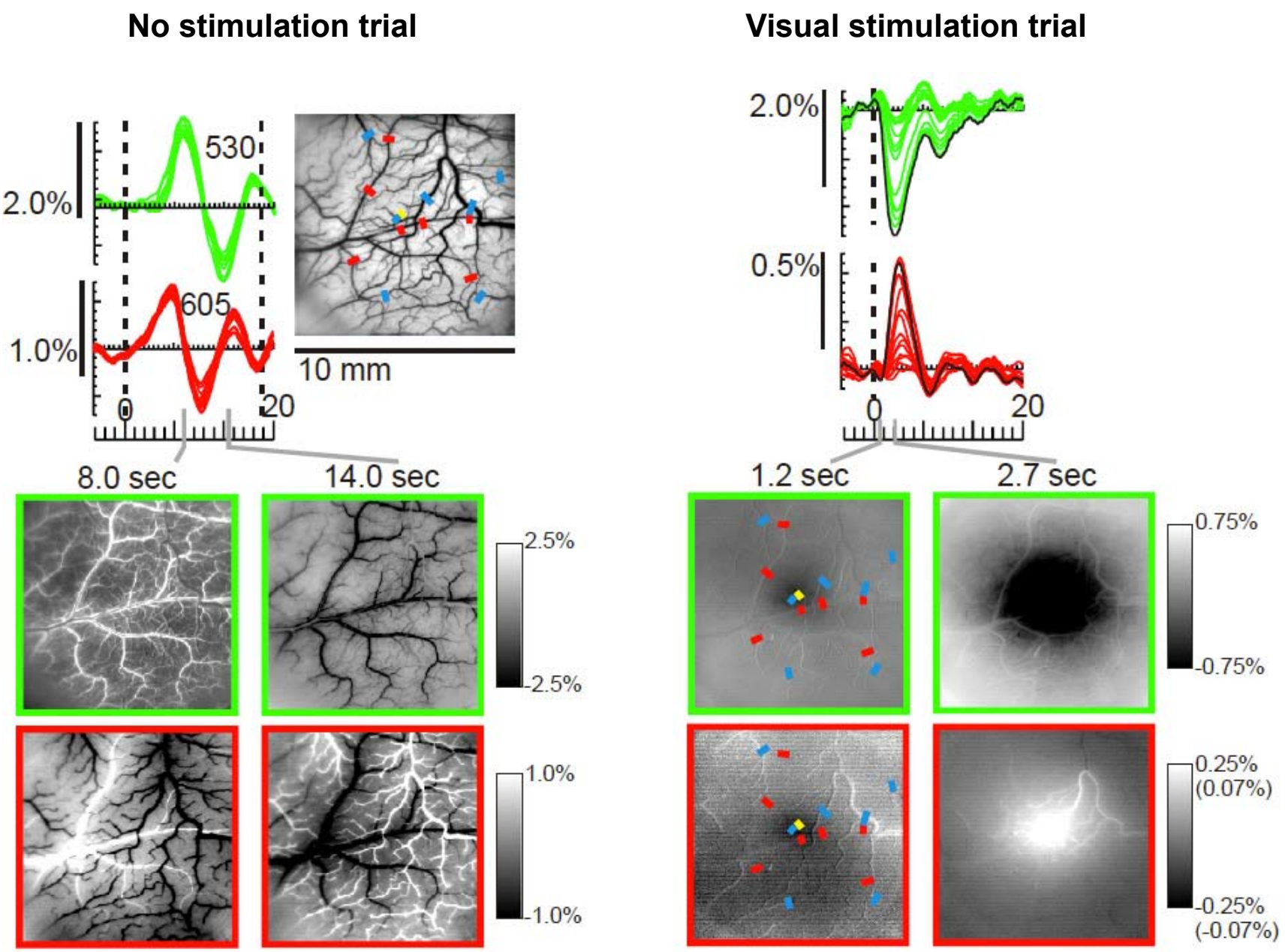
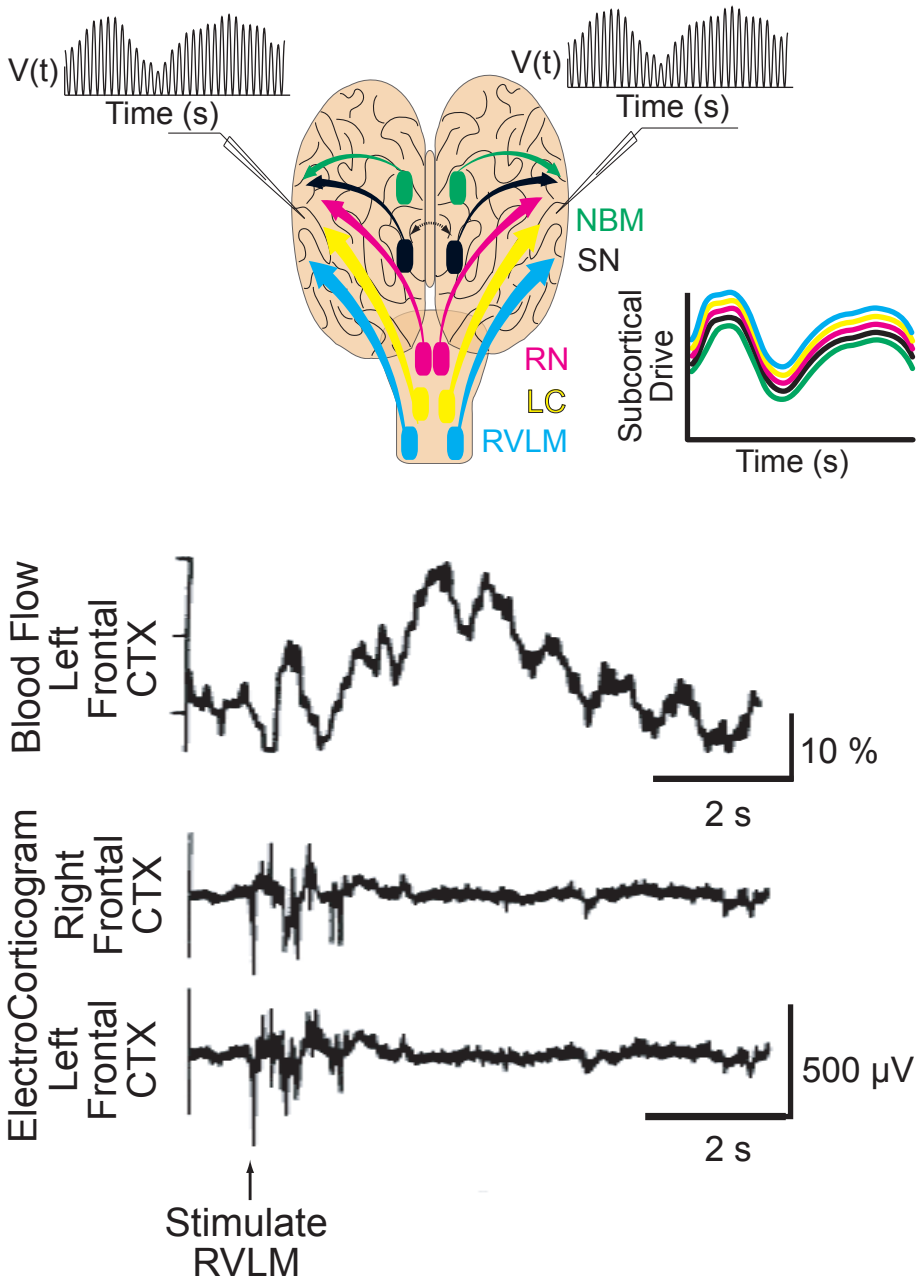


Sirotin & Das (Nature 2009)

Subcortical substrates for control of cortical blood flow and LFP power

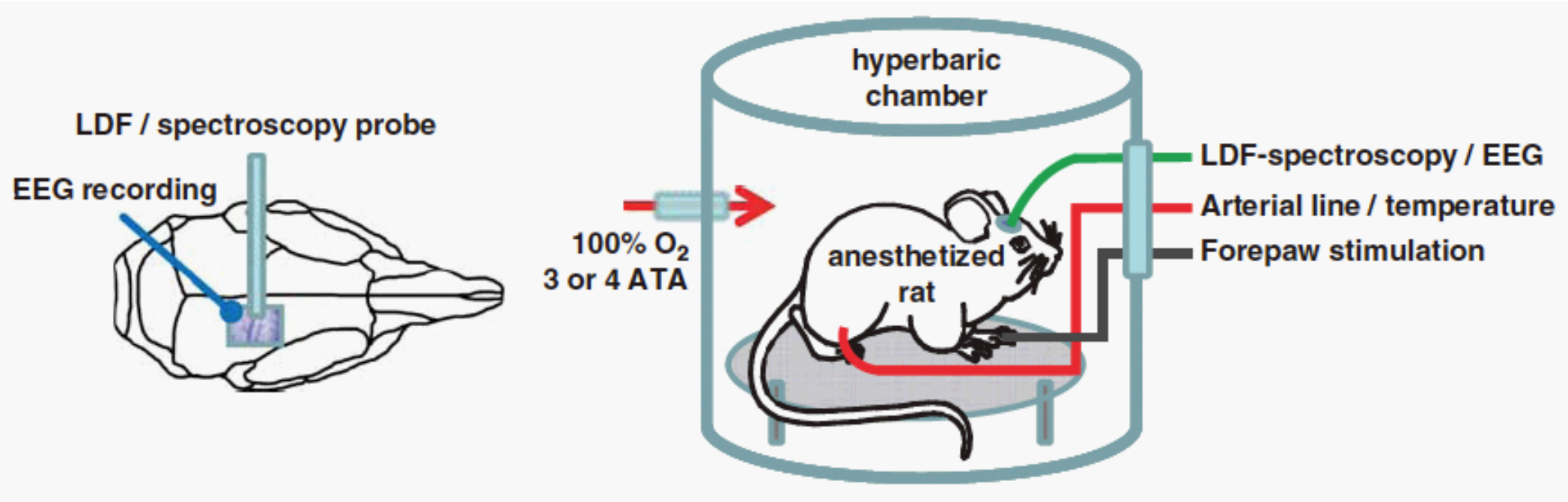


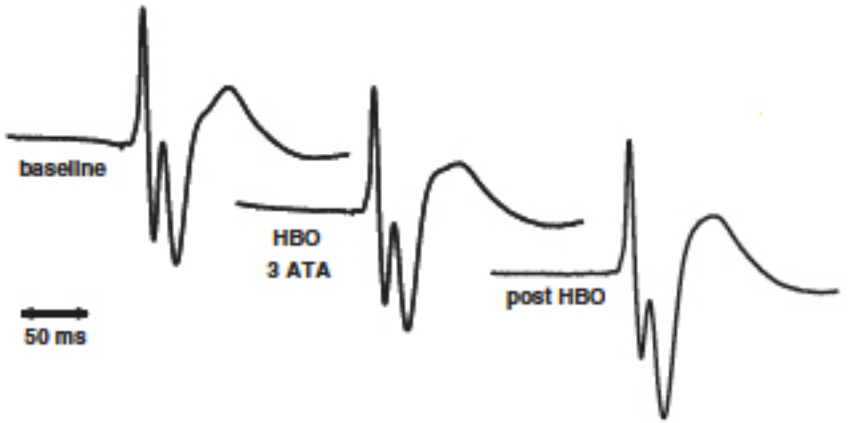
Drew, Duyn, Golanov & Kleinfeld (Nature Neuroscience 2008)

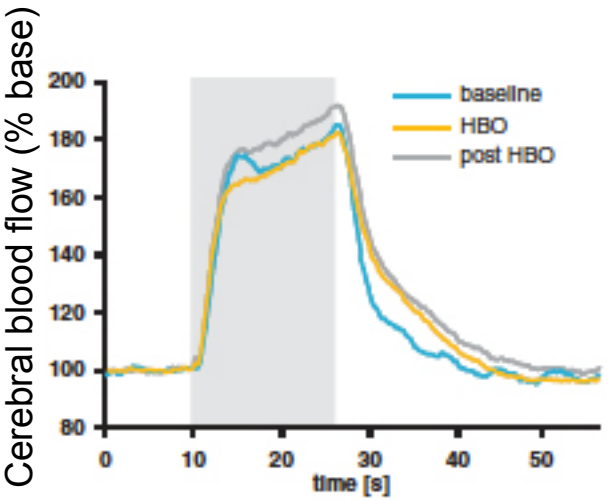
Lesson: Fluctuations in flow and potential subcortical variations to flow are as large as stimulus induced changes!

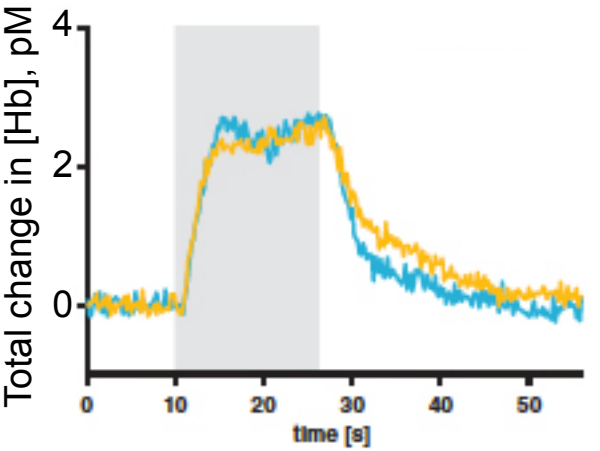
Question: Is the need for O_2, as occurs with stimulus driven neuronal activity, enough to trigger increased blood flow?

Hyperoxygenation (3 atmospheres) does not affect cerebral blood flow







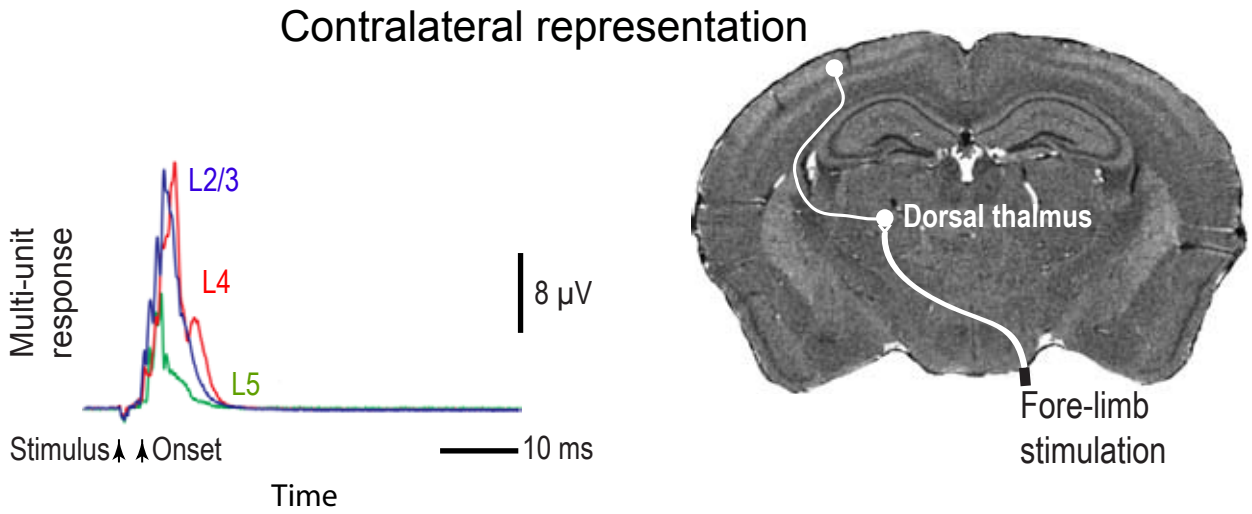


Lindauer, Leithner, Kaasch, Rohrer, Foddis, Fuchtemeier, Offenhauser, Steinbrink, Royl, Kohl-Bareis & Dirnagl (JCBFM 2010)

Question: Metabolites aside, does an increase in neuronal activity necessarily imply an increase in blood flow?

Neuronal activity changes blood flow - but logic remains to be deciphered

Example of changes in the lumen of surface arterioles for the two cortical hemispheres



Devor, Hillman, Tian, Waeber, Teng, Ruvinskaya, Shalinsky, Zhu, Haslinger, Narayanan, Ulbert, Dunn, Lo, Rosen, Dale, Kleinfeld & Boas (J Neurosci 2008)

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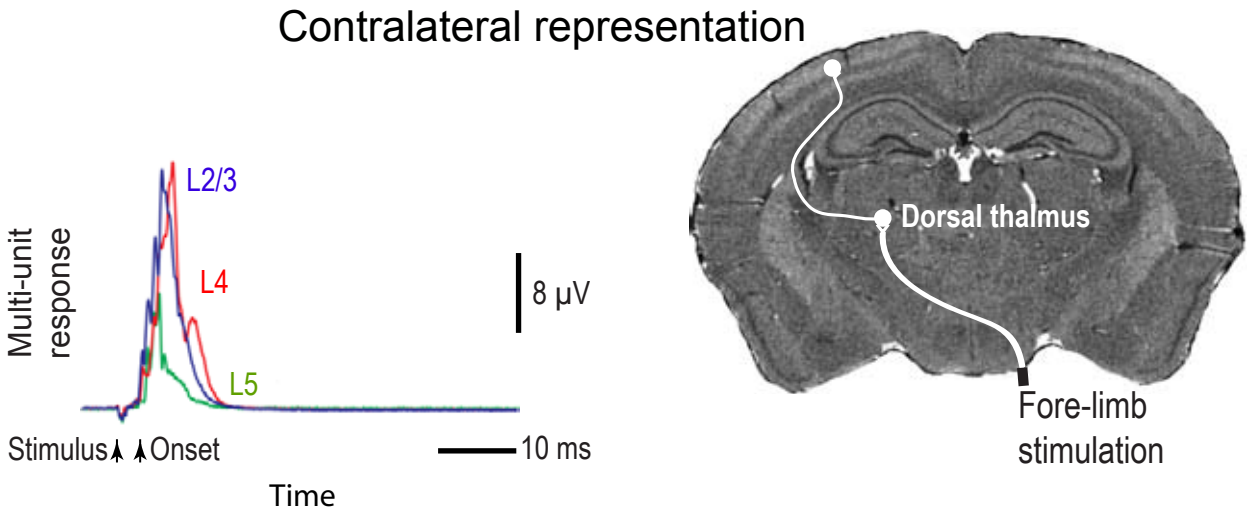

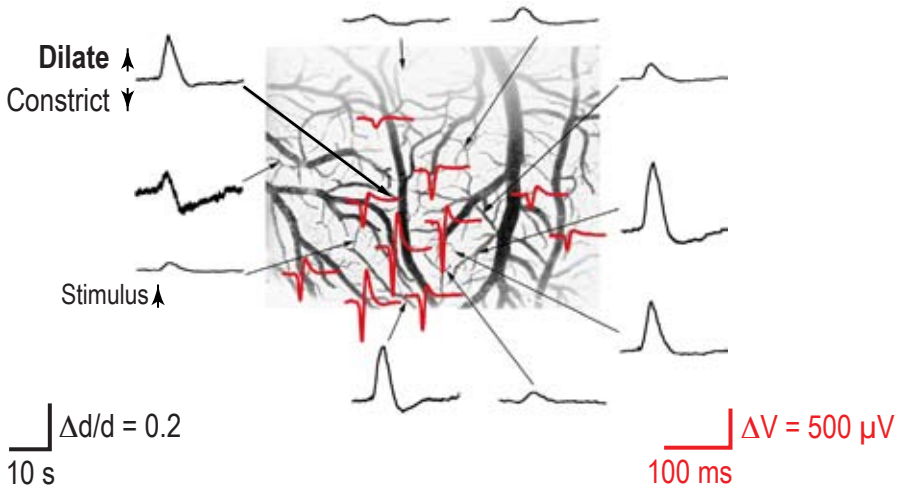

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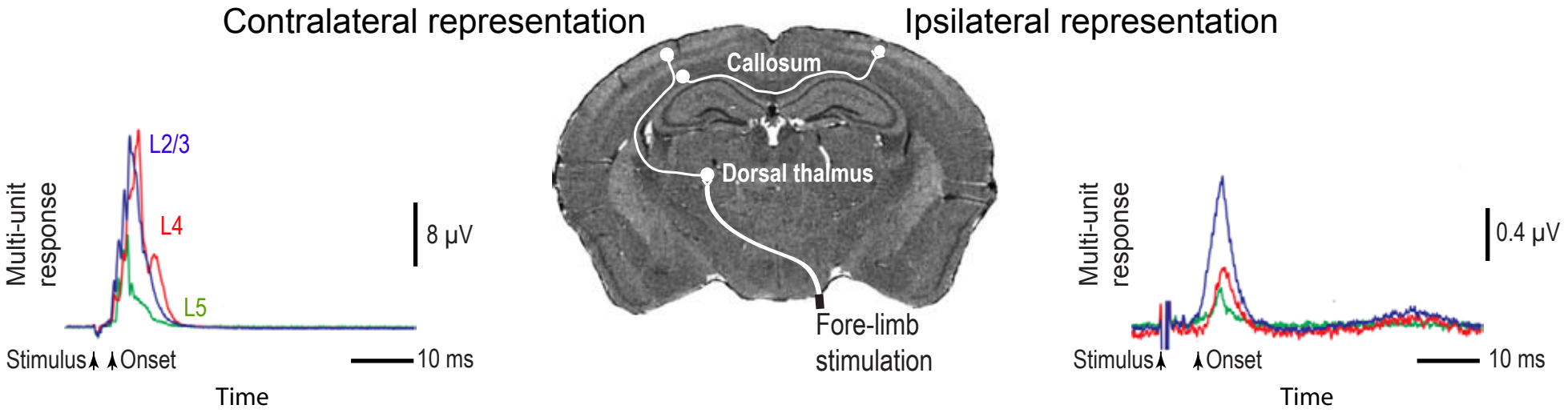

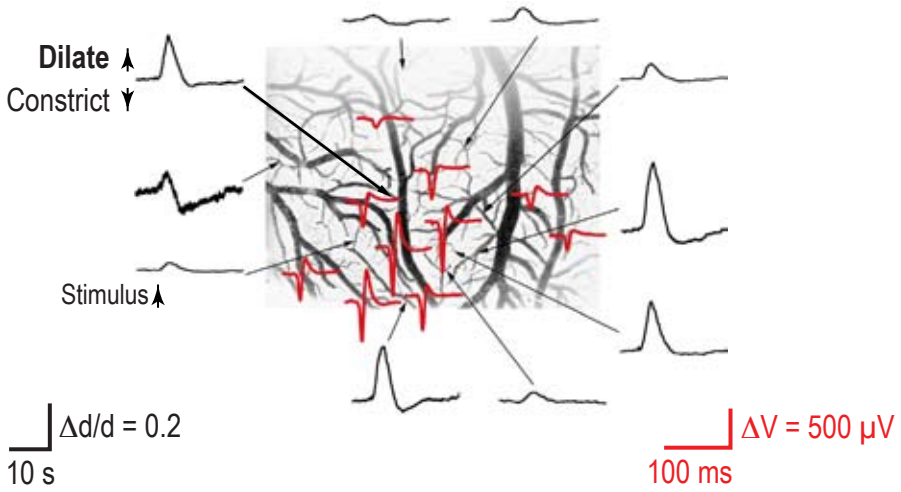

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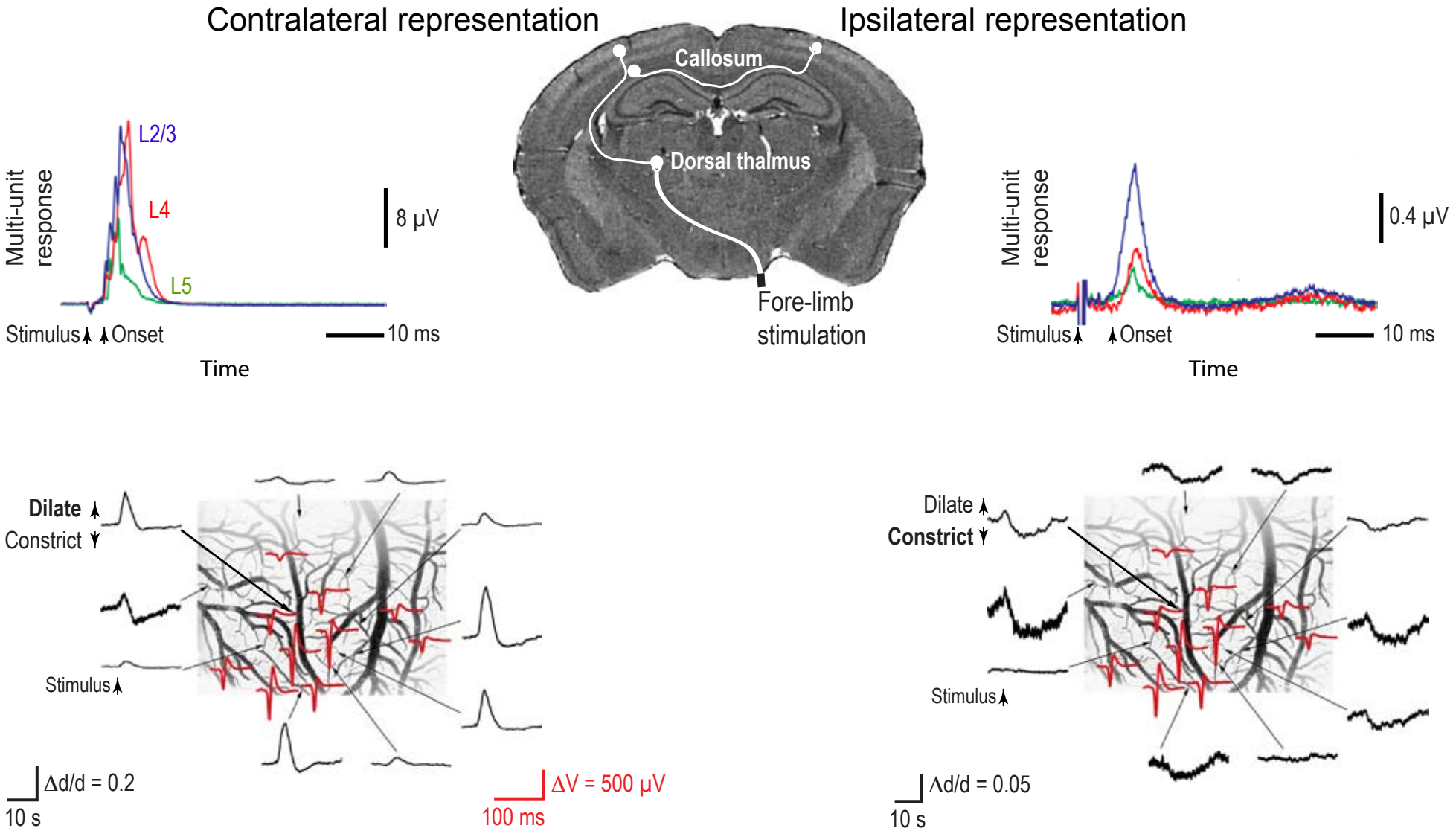
Neuronal activity changes blood flow - but logic remains to be deciphered

Example of changes in the lumen of surface arterioles for the two cortical hemispheres



Devor, Hillman, Tian, Waeber, Teng, Ruvinskaya, Shalinsky, Zhu, Haslinger, Narayanan, Ulbert, Dunn, Lo, Rosen, Dale, Kleinfeld & Boas (J Neurosci 2008)

Neural activity leads to competition between dilation and constriction

- Pyramidal activation of astrocytes leads to release of vaso modulators (e.g., Harder and McVicar laboratories)
- Interneuron release of neuropeptides (e.g., Hamel and Rossier laboratories)
- Extrinsic feedback from subcortical inputs, particularly from cholinergic and serotoninergic nuclei

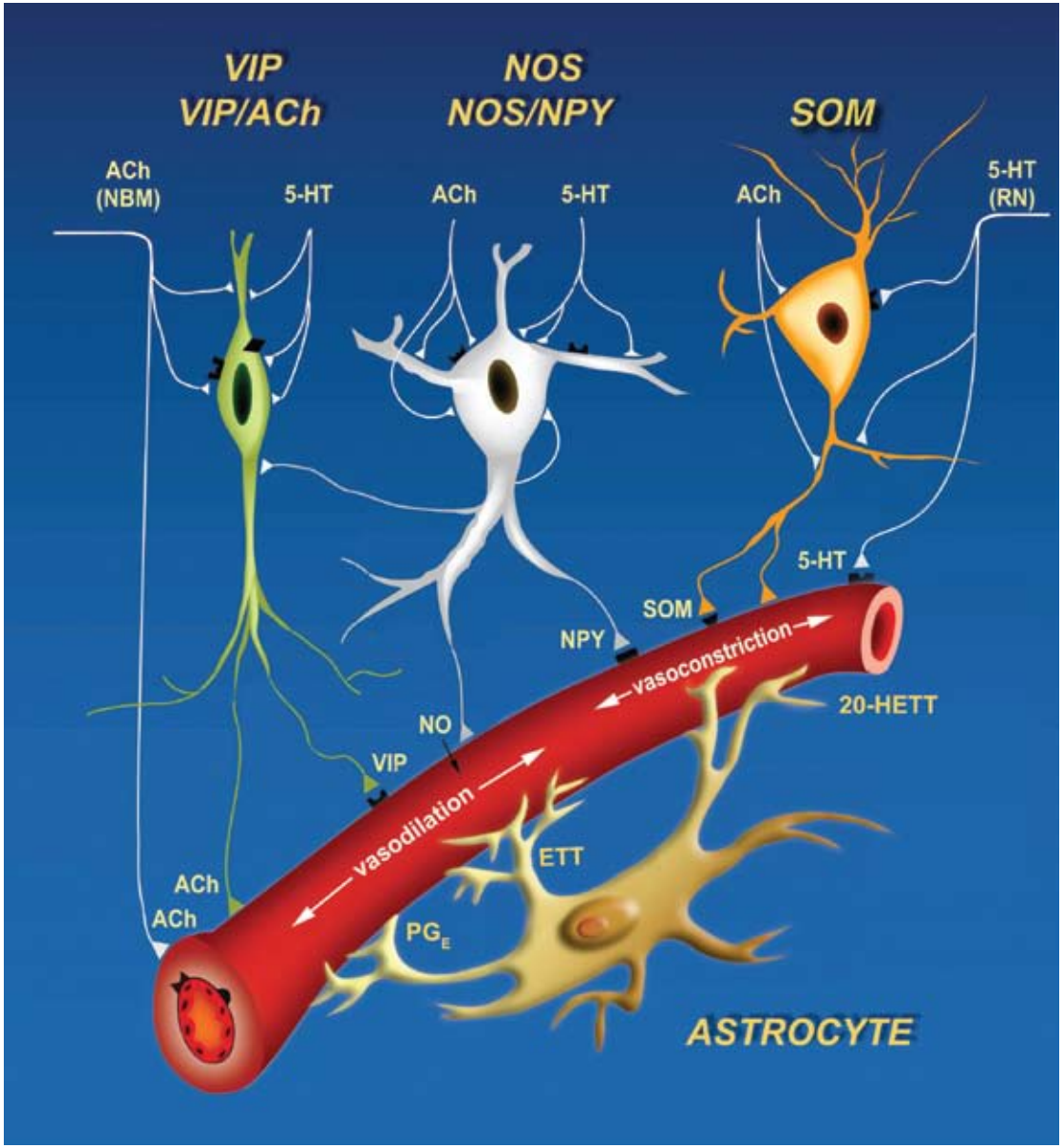
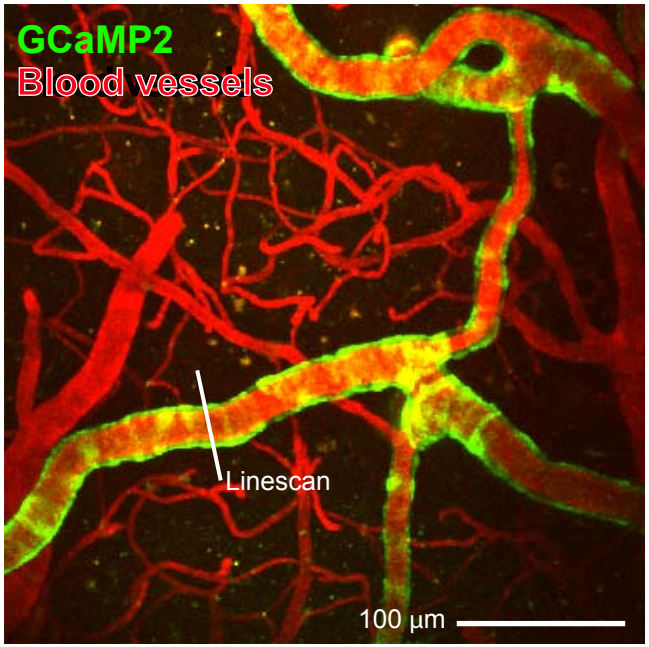
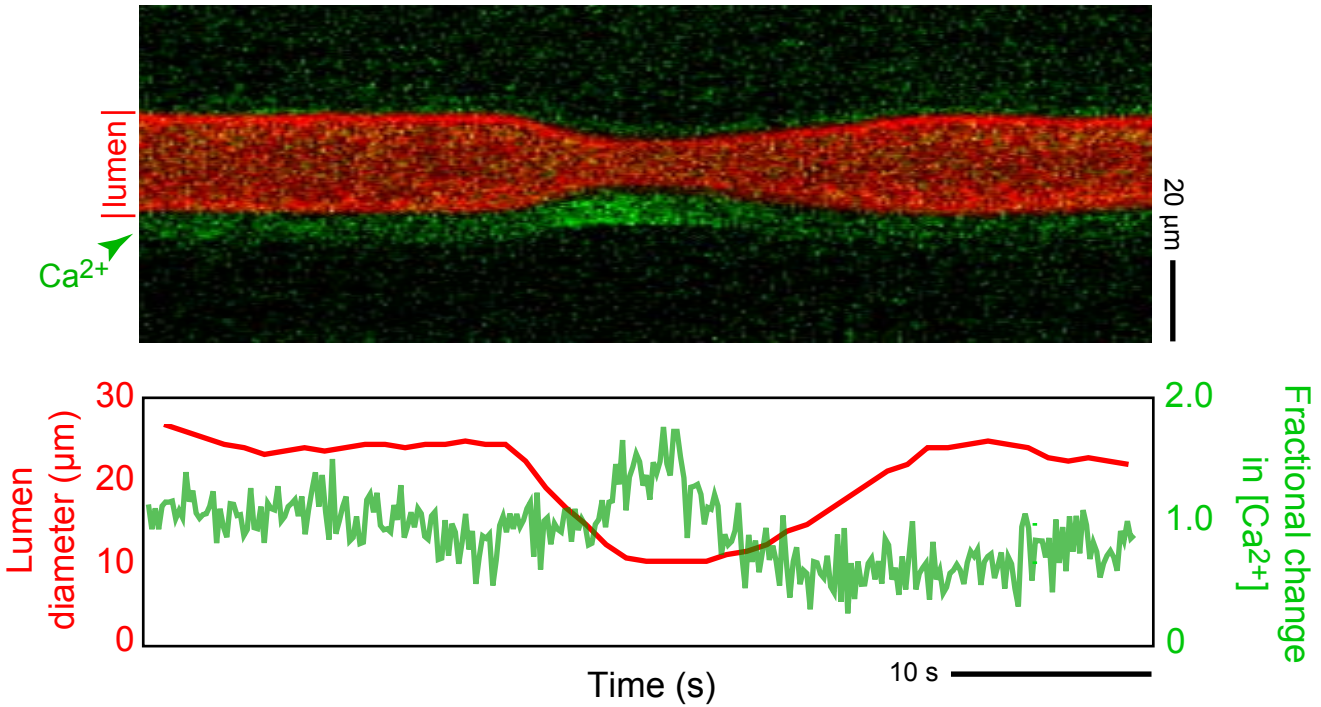


Figure adapted from Cauli, Tong, Rancillac, Serluca, Lambolez, Rossier & Hamel (J Neurosci 2004) and Iadecola & Needergard (Nat Neurosci 2007)

Smooth muscle activation may be imaged concurrent with blood flow

Example of measurements of muscle [Ca^{2+}] and lumen diameter in α-actin-BAC-GCaMP2 mice*





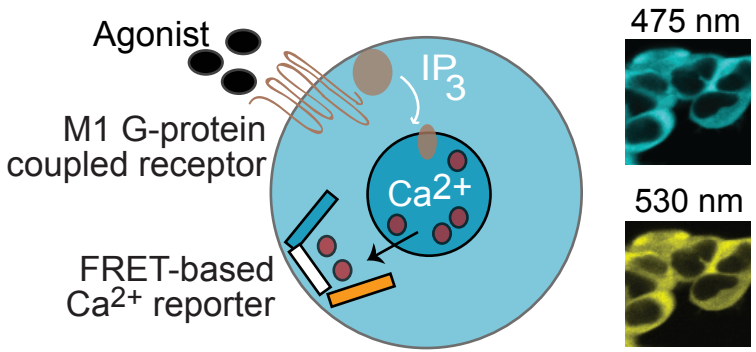
*Ji, Feldman, Deng, Greene, Wilson, Lee, Johnston, Rishniw, Tallini, Zhang, Wier, Blaustein, Xin, Nakai & Kotlikoff (JBC 2004)
Kleinfeld, Blinder, Drew, Driscoll, Muller, Tsai & Shih, (Front Neuroenergetics 2011)

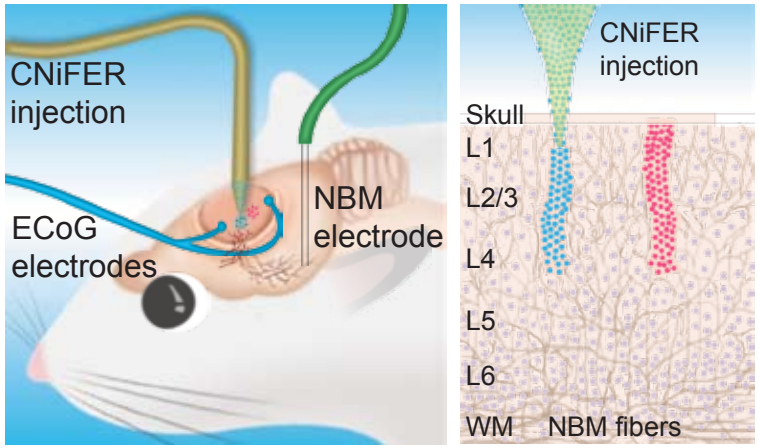
CNiFERs for *in vivo* detection of vasoactive signaling molecules

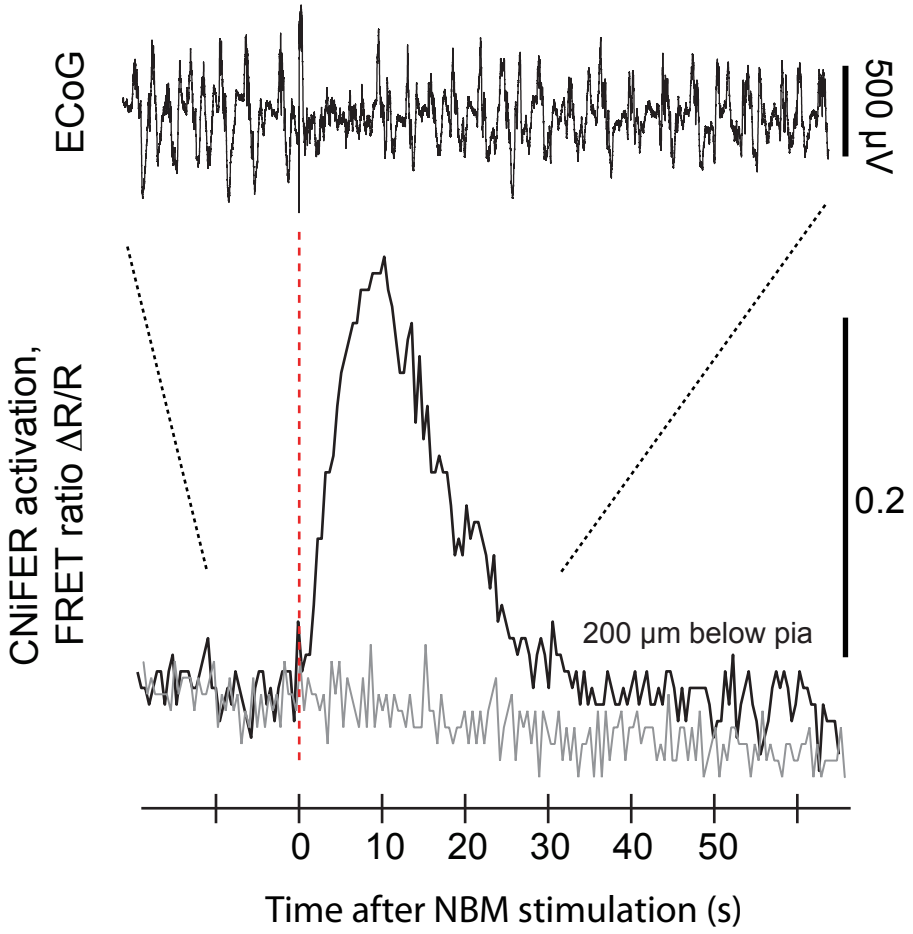
Vascular receptor	G-protein		Neuronal agent		Astrocyte agent	
	Pathway*	Action	Constrictor	Dilator	Constrictor	Dilator
Neuropeptide Y	$G_{i/o}$	cAMP ↓	■			
Somatostatin	$G_{i/o}$	cAMP ↓	■			
Acetylcholine (M5)	G_q	IP_3 ↑		■		
Serotonin	G_q	IP_3 ↑		■		
Vasointestinal peptide	G_s	cAMP ↑		■		
Endothelin (B)	$G_{i/o}$	cAMP ↓			■	
Adenosine (A2A)	G_s	cAMP ↑				■
Prostaglandin (E2)	G_q	IP_3 ↑				■

In situ neurotransmitter receptor activation monitored by cell-based neurotransmitter fluorescent engineered reporters (CNiFERs)

Example of extracellular ACh detection by M1-CNiFERs chronically implanted in rat

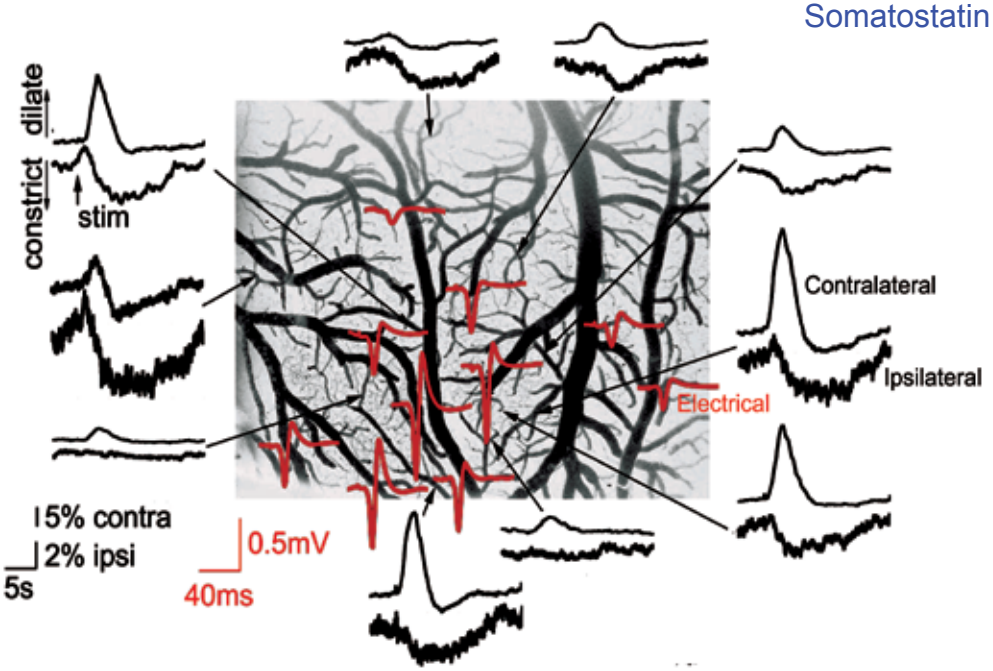






Nguyen*, Schroeder*, Mank, Muller, Taylor, Griesbeck & Kleinfeld (Nat Neuro 2010)

Example of a proposed experiment: SOM- and VIP-CNiFERs to detect change in balance of constriction versus dilation

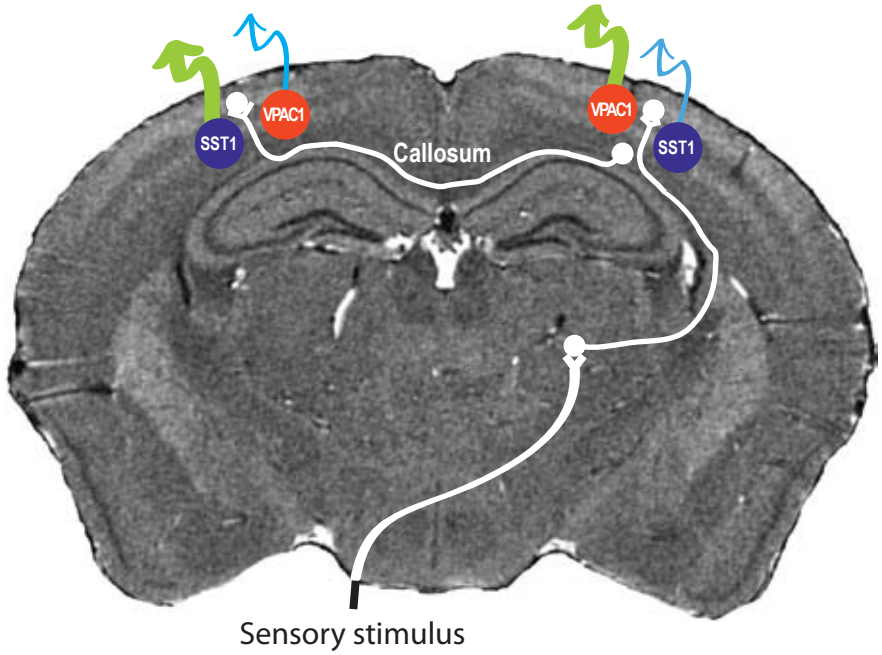


Vasoconstriction

Somatostatin activates SST1-CNiFERs

Vasodilation

VIP activates PVAC1-CNiFERs



Goal: Quantitative model to explain cortical blood flow dynamics

$$\textbf{Vascular output} \equiv f\left(\begin{array}{c}\text{Smooth muscle tension}\\ \vdots \\ \text{Vascular architecture}\end{array}\right)$$

$$\textbf{Smooth muscle tension} \equiv f\left(\begin{array}{c}\text{SOM+ inhibitory interneuron activation}\\ \text{VIP+ inhibitory interneuron activation}\\ \vdots \\ \text{Astrocyte activation} = f\left(\text{excitatory cell activation}\right)\\ \text{Extracortical and endothelial contributions}\\ \left[O_2\right]_{\text{tissue}}\end{array}\right)$$

Cortical angioarchitecture and vasodynamics

Thank you for your attention!